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Serological and Immunological Study of Toxoplasmosis in Heart Disease Patients

A thesis

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَإِنْ يَسْأَلْكَ اللَّهُ بَعْدَ ذَلِكَ فَاسْأَلْ لَهُ الْإِلَهَ هُوَ
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صدق الله العظيم
سورة الأنعام
الآية ١٧

Dedication

To : My Mother and Father with love
and gratitude's,

To: My wife,

To: My lovely daughter

Haider Sabah Abdullhussein

Acknowledgments:

First of all, I would like to thank the merciful Allah for helping me in completing this work. By principles of Allah, I catch to complete this study .

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Haider

Summary

Toxoplasmosis is the general term for infection and disease in human and animal caused by a parasite called *Toxoplasma gondii*, this disease is prevalent among different categories of people, Heart disease patients are one of them. Because of the dormancy status of *T. gondii*, there is ambiguity and variation in the immunity of patients. This study aimed to investigate the immune status of heart disease patients infected with toxoplasmosis, to detect the prospective relationship between heart disease patients and *T. gondii*, and investigate the relationship between toxoplasmosis and risk factors which included gender, age, blood group, contact with cats, and habitation.

One hundred fifty samples of blood had been collected at period from August 2016 till November 2016 from patients with heart disease, they lying or visit Heart Medical Centre in Thi-Qar province. According to gender, the patients divide in to (81 males and 69 females) with age ranging 18 to 82 years. Also one hundred samples of blood had been collected from apparently healthy persons as negative control, dividing according to gender in to (55 males and 45 females) with age ranging 19 to 56 years. All samples of both subjects had been tested by ELISA technique to detect anti-*Toxoplasma* Abs (IgG & IgM). While the positive samples with toxoplasmosis were tested to detect the level of interleukins (IL-10, IL-12 and IL-17).

The overall of seropositivity of anti-*Toxoplasma* Abs that scored by ELISA in the current study was 44% in heart disease patients are higher than those in apparently healthy controls 19%, and there was a significant difference between them. There is a significant difference between male and female, in the seropositivity of anti-*Toxoplasma* Abs that scored in controls, in male was higher than those in female. Presence of anti-

toxoplasmosis Abs in all types of heart disease patients with significant difference among them, and the highest percentage of anti-toxoplasmosis Abs in Mitral stenosis(MS) was 55.8% , and the lower value was 30.3% in Myocardiopathy.

There are significant differences between the age groups and the percentage of anti-*Toxoplasma* Abs in heart disease patients with highest value was 63.2% at age group of 61-75 years, and the lowest one was 26.3% at age group of 15-30 years. While no significant differences between the age groups and toxoplasmosis in apparently healthy controls with highest value was 19.5% at age group of 31-45 years, and the lowest one was 8.6% at age group of 15-30 years.

The results demonstrated that seroprevalence of toxoplasmosis was significantly associated with close contact with cats. The percentage of seroprevalence anti-*Toxoplasma* Abs in heart disease patients and controls inhabited rural area was 52.5% and 32% are higher than those inhabited urban area was 34.3% and 6% respectively. Some blood parameters were estimated of both subjects infected and non-infected with toxoplasmosis. There was high significant difference at $p \leq 0.05$ observed in mean ratio of eosinophil's number between infected and not infected subjects with toxoplasmosis in both subjects. While there was no significant differences in mean ratio of total count of WBCs. Moreover, mean of lymphocytes number recorded significant differences between infected and non -infected heart disease patients only. The blood group phenotypes of the infected heart disease patients and controls highlight the role in toxoplasmosis infection and the AB blood group characterized by the highest significant percentage of infection.

The results of interleukins appeared high mean levels of IL-12 82.85 pg/ml in heart disease patients infected with toxoplasmosis when compared with control 60.97 pg/ml, and there was significant difference

between them. While there was no significant difference in IL-10 and IL-17 in both patients and controls. The results also showed decreased level of IL-17 in patients with heart disease. The increased level of IL-12 in patient with heart disease infected with toxoplasmosis indicate to the activity of cell mediated immunity in those patients and may be lead to provoke an inflammatory status in the heart valves and muscle then worse heart disease to be sever and need complicated treatments.

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List of Abbreviations

Abbreviations	Description
AHT	Arterial hypertension
APCs	Antigen presenting cells
CAD	Coronary artery disease
CDC	Centers for disease control and prevention
CHD	Coronary heart disease
CBS	Cat parasite schizophrenia
CD	Cluster differentiation
CFT	Complement fixation test
CMV	Cytomegalovirus
DC	Dendritic cell
ELISA	Enzyme linked immunosorbent assay
EIA	Enzyme immuno assay
GBD	Global burden of disease
GM-CSF	Granulocyte macrophage stimulating factor
GTPases	Guanosin Triphosphatase
Hb	Hemoglobin
HRP	Horseradish peroxidase
HIV	Human immunodeficiency virus
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HF	Heart failure
HTNF α	Human tumor necrosis factor-alpha
KD	Kilo Dalton
IHD	Ischemic heart disease
IgG	Immunoglobulin class gamma
IgM	Immunoglobulin class Mu
IgA	Immunoglobulin class Alpha
IgE	Immunoglobulin class E
IL	Interleukin
IFN- γ	Interferon-gamma
IHAT	Indirect heamagglutination test
IFA	Indirect Fluorescent Antibody Assay
iNOS	Inducible Nitric oxide synthase
ICAM-1	Intercellular adhesion molecule-1
iNKTS	invariant Natural Killer T cells
LAT	Latex agglutination test
MAT	Modified agglutination test

List of Abbreviations

Abbreviations	Description
MHC	Major histocompatibility complex
μM	Micrometer
NO	Nitrogen Monoxide
NK	Natural Killer
NYHA	New York Heart Association
PRRs	Pattern recognition receptor
PAMPS	Pathogen association molecular patterns
PVM	Parasitophorous Vacuoles Membrane
PV	Parasitophorous Vacuoles
Pg	Picogram
PCR	Polymerase Chain reaction
ROI	Reactive Oxygen Intermediate
SPSS	Statistical package for social science
STAS	Signaling transduction activator transcription
TCR	T-Cells Receptor
TE	Toxoplasmic Encephalitis
Thp	T helper precursor
TLR	Toll-like receptors
TL	T-Lymphocyte
Th	T-helper

Chapter

One

Introduction

1.1 Introduction:

Toxoplasmosis is a coccidian and cosmopolitan disease in humans and animals produced by the opportunistic parasite named *Toxoplasma gondii*, it is caused various disease for example toxoplasmic encephalitis and congenital birth deficiencies. (Zhou *et al.* , 2011) . Toxoplasmosis can reason grave pathological lesions for example hepatitis, blindness, abortion, pneumonia, and sever neurological disorders, these categories of diseases are seen chiefly in people with weak immune systems (Hill and Dubey,2002). *T.gondii* is an obligate intracellular protozoan parasite, which can influence all warm-blooded vertebrates and feathered creatures all through the world, including individuals (Webster, 2010). In the normal conditions the immune system is capable to control parasite and persist as infection. However, some tachyzoites seepage the immune system, convert back to bradyzoites cysts in brain and muscular tissue to create a life-long chronic infection. In healthy individuals, the infection is usually asymptomatic, but reactivated infection in immunocompromised patients can lead to toxoplasmosis, and acute infection while in pregnant can cause harm to the developing baby(Black and Boothroyd , 2000).

Humans may persist infected for life and will stay asymptomatic unless immunosuppression occurs (Herrmann *et al.*, 2010). The infection has a worldwide distribution, around 33% of the total populace is infected with *T. gondii*. (Hill *et al.* , 2005), however the seroprevalence differs extensively between nations from 10% to 90%) (Rosso *et al.* , 2008). The prevalence of *T. gondii* has been reported to increase with age (>forty years) and in women consuming raw meat, fruits and vegetables (Nijem and Al-Amleh, 2009). Cats and other members of felidae are the definitive hosts, whereas human and wide range of animals, birds and rodents act as intermediate hosts (John and Petri , 2006). After the acute stage, the cyst forms of *T. gondii* found in many organs, espically the brain, heart and skeletal muscle, so establishing a chronic infection (Waree , 2008). The diagnosis of

toxoplasmosis is usually by identification the Immunoglobulin, for example, IgG, IgA and IgM antibodies in the serum tests of patients utilizing strategies Enzyme linked immunosorbent assay test (ELISA), Latex agglutination test (LAT), Indirect immunofluorescent test (IFT) (Dubey, 2010).

The first occurrence of toxoplasmosis was recorded in 1938 by Machattie, through histological isolation of the parasite from spleen and lungs of two stray dogs in Baghdad (Al-Saad, 2013). Recent study by AL-Mosawi, (2014) recorded that the rate of toxoplasmosis in Thi-Qar province among universities students were 21.94% by using ELISA test (AL-Mosawi, 2014). *T. gondii* have ability evade host immune system escapes primary from macrophage killing apparatus then infect it. Infected macrophage is also considered as aim for T cell and that happens because condensed expression of major histocompatibility (MHC) class II, also makes the production of counter regulatory molecules by infection like cytokines or Interleukins. That defend host by regulating a possibly inflammatory response, also it will impede cytokines produced through Th1 and antimicrobial activity of macrophage (Aliberti, 2005; Ali *et al.*, 2012).

The major elements play role in immune response to parasitic infection were Natural killer cells, T lymphocytes and Macrophages in combination with cytokines. Cytotoxicity and secretion of cytokines particularly IFN- γ consider as T cells role. Production of IL-12 and IFN- γ is essential to control infection by *T. gondii* which are a part of cell-mediated immunity the mechanism focused on limiting the parasite reproduction during the acute phase of infection (Cordeiro, 2008). Earlier researches have revealed that most cytokines produced by neutrophils, macrophages and dendritic cells (DCs) like interleukin-12 (IL-12) in infected animals, was liable for the initiation of T helper 1 (Th1) cell responses and *T. gondii* clearance (Gazzinelli *et al.*, 1993; Reis *et al.*, 1997; Yap *et al.*, 2000). IL-17 has been implicated in the pathology associated with many forms of infection-induced inflammation (Intlekofer *et al.*, 2008), it has also been

linked to resistance to multiple pathogens, including *T. gondii*. (Kelly *et al* .,2005). The early induction of neutrophil induction during *T. gondii* infection is dependent on IL-17-mediated signaling as chemotactic factor. The neutrophils clear the parasites during initial stages of infection so that adaptive immunity, which is induced later, is not overburdened. In the presence of suboptimal levels of neutrophils, parasite load is not efficiently reduced and adaptive immunity is unable to handle this elevated burden, which ultimately leads to increased mortality (Yisong and Richard ,2009) .

1.2 The aims of this study :

- 1 - Estimate the prevalence of anti-*Toxoplasma* Abs (IgM & IgG) between patients with heart disease those admission or visit Heart clinical center in Thi-Qar province by using ELISA .
- 2- Investigate the relationship between toxoplasmosis and risk factors which included gender, age, blood group, contact with cats, and habitation.
- 3 - Investigate the role of interleukins IL -10 , IL- 12 and IL- 17 in patients with heart disease those have anti-*Toxoplasma* Abs and compare that with apparently healthy controls.

Chapter

TWO

Literature Review

2.1 Historical review :

The first description of *Toxoplasma gondii* was by Nicolle and Manceaux in 1908, they noticed it in North African rodents *Ctenodactylus gondii* in the same time it was described in Brazil in rabbits by Splendore (Ferguson, 2009). In 1909 the protozoan disease is differentiated from *Leishmania* and called as *T. gondii* (Sukthana, 2006). The genus name '*Toxoplasma*' is derived from the Greek word 'toxon' meaning bow, which describes the crescentric shape of the tachyzoite stage, and the species name '*gondii*' from the rodent from which it was first isolated (Markus, 2003). In 1923, Junku observed the cyst in the retinal tissue of a child with hydrocephalus and chorioretinitis in a circumstance of congenital toxoplasmosis but he did not separate the organism (Cox and John-Alde, 2005). In 1927, Torres reported the occurrence of *Toxoplasma* in muscles, cardiovascular and subcutaneous tissues in humane as well as the nervous system, also in 1936 Richter discussed the role of *Toxoplasma* as a cause of neonatal meningo-encephalomyelitis (AL-Mosawi, 2014). Sabin and Olitsky (1937), described the clinical symptoms of human toxoplasmosis. Nevertheless, successfully isolated the parasite from tissue from a neonate with encephalitis, by animal inoculation when Wolf *et al.*, in 1939 noticed the parasite in children with encephalomyelitis (Cox, 2002).

Although Sabin and Feldman (1948) described the dye test, which is based on complement mediated cytolysis of Ab-coated live *T. gondii* tachyzoites, which is indicated by their inability to take up methylene blue. It is still the reference method for the serodiagnosis of toxoplasmosis (Reiter-Owona *et al.*, 1999). It is the "gold standard" test, which is a highly specific and sensitive test, but its main disadvantages are its high cost and the human hazard of using live organisms (Dubey, 2002). Also, Frenkel introduced the *Toxoplasma* skin test (Gillespie and Pearson, 2001). which includes the intradermal injection of

soluble antigen (toxoplasmin) from a pathogen. The positive test means past infection with that pathogen, but the negative result does not always indicate the absence of infection or antibodies (Roitt *et al.*, 2001). In 1952 Wilder surely could demonstrate *T.gondii* in tissue section in adult eyes (Montoya and Remington, 2000). Found in analysis of uveitis instances Smith and Ashton (1955) found that the higher percentage of their patients demonstrated antibodies against *T. gondii* than the normal human population and Goldman *et al.*, (1957) used the immuno- fluorescent microscope to analyse a person's immune response against *T. gondii* (Alcamo, 1997). Remington and Kaufman (1960), isolated *Toxoplasma* parasite from a person's necrotic uterus and hysterectomy cases. In 1970 sexual cycle of *T. gondii* was elucidated by Frenkel (Tenter *et al.*.,2000).

2. 2 Morphology of *T. gondii* :

It has three infective stages including the motile and quickly dividing tachyzoites during an acute infection , sporozoite stage in oocyst and bradyzoite stage that slowly dividing during chronic infection (Jones *et al.*, 2001b).

2.2.1 Tachyzoite

Tachyzoite is often crescentic in shape, approximately 6µm long and 2µm wide (Bhopale, 2003) . The posterior end is often round while the anterior end is pointed (Hill *et al.* ., 2005) . Tachyzoite was surrounding by coat called pellicle which is consist of three membrane called a plasmalemma and an inner membrane consist of two membrane (Bhopale, 2003).They are consist of various organelles such as rhoptries, conoid, mitochondria, golgi complex, apical and polar rings, micronemes, micropores, subpellicular, microtubules, ribosome , and a centrally located nucleolus (Dubey *et al.* ., 1998). The conoid is often located at the anterior end (polar end), which is consist of six to eight

fibriall elements, this structure have chief role because it is associated with the penetration of the parasite during the membrane of the host cells. Ending within the conoid are the rhoptries, these are four to ten gland like structures with an anterior narrow neck and posterior-sac-like end reaching as far as the nucleus. When the parasite has attached to the host cell, their contents are cleared through the conoid. The micronemes are tube like structures at the anterior end of the parasite. They are usually less than 100 placed at the conoidal end of the parasite and are also have role in invasion of the host cell. The micropores are sites specialized for the acceptance of nutrients during endocytosis (Black and Boothroyd, 2000), as show in figure (2-1).

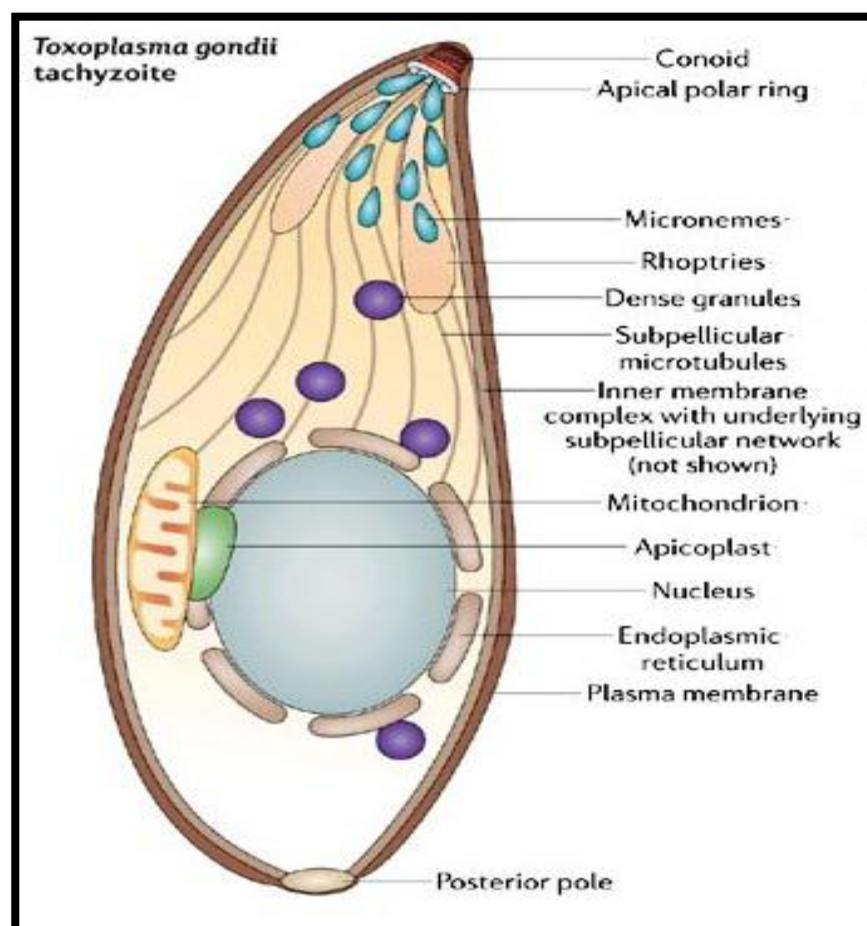


Figure 2-1 : Tachyzoite of *Toxoplasma gondii* (James *et al.* , 2001).

2.2.2 Bradyzoites and tissue cyst

Bradyzoites result from the transformation of tachyzoites into a slow-dividing phase and procedure tissue cysts. These cysts are elongated in muscular cells and more or less spheroid in brain cells. They differ in size from 10 μm for the younger cysts, composed of only two bradyzoites, to up to 100 μm for the older ones, composed of hundreds or thousands of densely packed bradyzoites (Robert-Gangneux and Dardé, 2012). Although tissue cysts may develop in visceral organs, including the lungs, liver and kidneys, they are more prevalent in the neural and muscular tissues, including the brain, eyes, and skeletal and cardiac muscles. Bradyzoites appear structurally similar to tachyzoites through light microscopy, but ultra-structurally by electron microscopy bradyzoites have a more honeycombed rhoptries, more posterior nucleus than tachyzoites, and comprise amylopectin granules (Weiss and Kim, 2011). A distinctive morphology of bradyzoites is that their parasitophorous vacuole becomes thickened forming the tissue cyst wall.

The cyst wall is rich in carbohydrate and stains with various lectins (Zhang *et al.*, 2001). They are more slender and less susceptible to destruction by proteolytic enzymes than tachyzoites. Bradyzoites have a latent metabolism, well adapted to long-term survival. The death of the host cell may trigger the disruption of the cyst wall and the consequent liberation of bradyzoites. The resistance of bradyzoites to the acid pepsin (1 to 2 hrs survivals into pepsin-HCl) allows their transmission through ingestion. Intact tissue cysts probably do not cause any harm and cysts remain intracellular throughout their life span of the host (Robert-Gangneux and Dardé, 2012). Modifications of the environmental pH, shifting the temperature, IFN- γ treatment, or the inhibition of the mitochondrial respiratory chain induces transition of tachyzoite to bradyzoite stage (Tomavo, 2001). It is believed that tissue cysts can

periodically rupture thus releasing parasites that re-invade host cells and establish new tissue cysts (Reiter-Owona *et al.* , 2000).

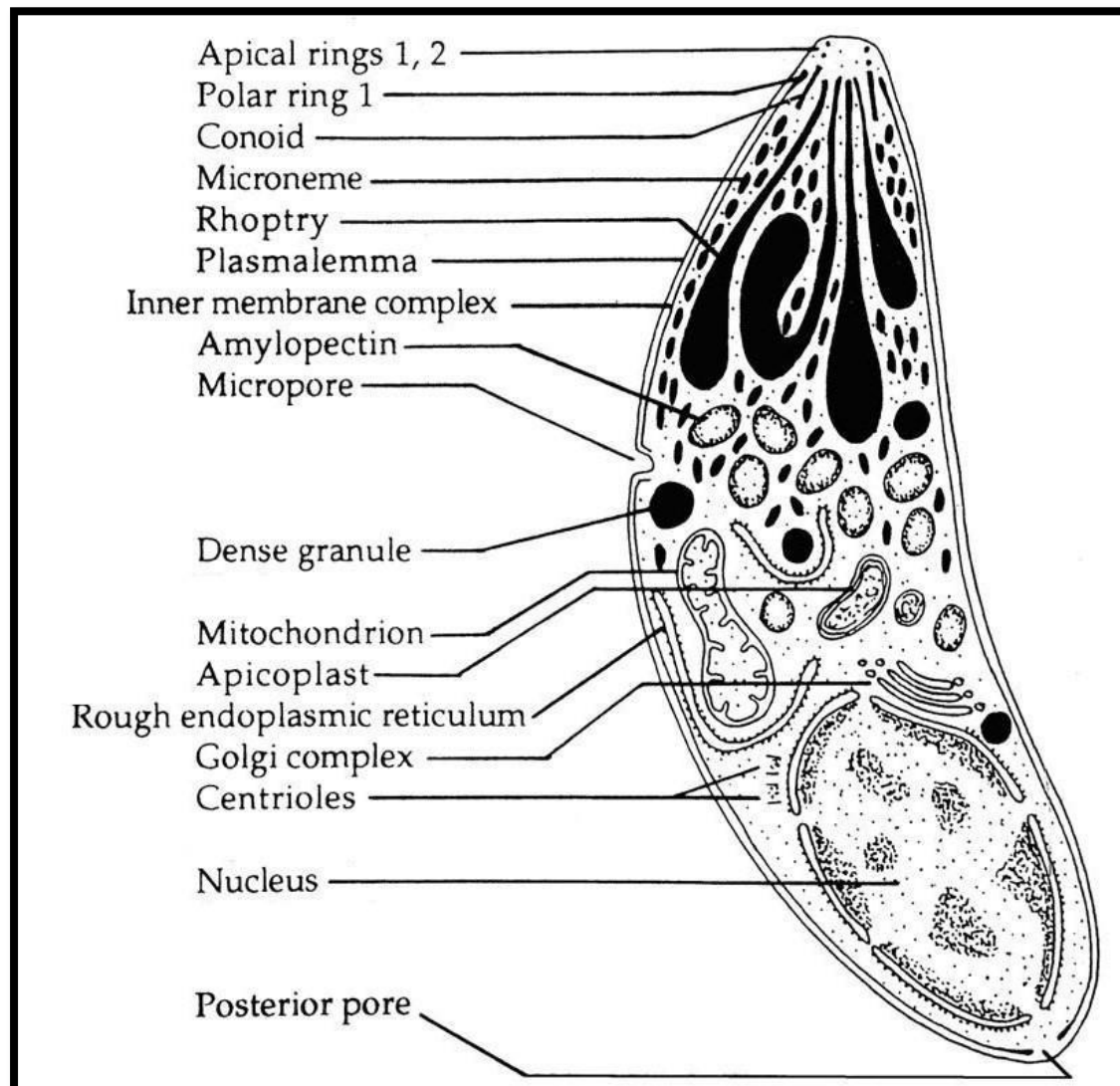


Figure 2-2: Bradyzoite of *T.gondii* (Dubey *et al.*,1998).

2. 2. 3 Oocyst

Unsporulated Oocysts are often spherical to subspherical with 10x12 μm in diameter. While sporulated Oocysts are ellipsoid to subspherical with 11x13 μm in diameter, as show in figure(2-3). Each sporulated Oocyst encompasses two sporocysts which are usually 6x8 m. Whearas each sporocyst encompass 4 sporozoites, which are usually measuring 2 x 6 - 8 μm . Thus, the sporulated

oocysts have eight sporozoites. The wall of sporulated oocysts involve of three layers. One electron-dense outer layer, an electron-lucent middle layer and a moderately electron-dense inner layer (Dubey *et al.*, 1998). Oocysts sporulation depends on the temperature and oxygen and may take 1 to 21 days. Sporulation happens in 2 to 3 days at 24°C, 5 to 8 days at 15°C and 14 to 21 days at 11°C. Sporulation does not take place below 4°C or above 37°C. Sporulated oocysts of *T. gondii* are very resistant to environmental conditions. They survive short periods of dehydration and cold and remain infectious in moist soil or sand for up to 18 months. They are highly impermeable and, therefore, are also very resistant to disinfectants (Frenkel, 2000) .



Figure 2-3: Unsporulated Oocyst (A) and Sporulated Oocyst (B) (Blagburn, 2010)

2.3 Life cycle of *T. gondii*

Includes definitive and intermediate hosts, the sexual cycle of the parasite can take place in the digestive tract epithelial cell of the cat (both intra-intestinal and extra-intestinal life cycle occur in cats), but in the intermediate host only an asexual cycle occurs (Dubey, 2008). When an intermediate host ingests oocysts, sporozoites are released into the gut lumen and pass through the gut epithelium to enter cells in the lamina propria. In case an intermediate host

ingests tissue cysts the released bradyzoites behave similarly to sporozoites in which upon ingestion both sporozoites and bradyzoites invade the intestinal epithelium, differentiate into the rapidly growing tachyzoite form and disseminate throughout the body, both sporozoites and bradyzoites transform into tachyzoites that enter a host cell where they divide rapidly until the cell bursts (Weiss and Kim, 2011). Tachyzoites can infect virtually any nucleated cell type, although a tropism for certain cell types (for instance retinal vascular endothelial cells) has been reported (Smith *et al.*, 2004). After invading a cell, the tachyzoites can rapidly divide and after death of the infective host cell they will invade adjacent cells or after traveling through the blood stream attach to cells elsewhere in the body.

The tachyzoite stage can transform into a slowly dividing bradyzoite (Klaren and Kijlstra, 2002) because as immunity develops, replication of tachyzoites decreases and tissue cysts develop which do not normally produce a host reaction (Saavedra, 2003). Cats release Oocysts with their faeces and play an important role in the epidemiology of toxoplasmosis (Bayarri *et al.*, 2012). Cats acquire the infection via consumption of tissue cysts present in the organs/tissues of a chronically infected intermediate host prey (such as mice) or via ingestion of Oocysts within their food or water. After ingestion of tissue cysts the cyst wall is digested by gastric acid, bile and lytic enzymes of the upper digestive tract, which results in releasing bradyzoites and the released bradyzoites, invade the intestinal epithelium. Besides systemic dissemination after conversion to the invasive tachyzoite stage, some organisms inside the epithelium of definitive host undergo five different developmental stages that reproduce asexually by endodyogeny, where two daughter cells are created inside one and by schizogeny to differentiate into micro and macro gametocytes within 2 days of infection and involving the formation of multiple merozoite cells around a previously divided nucleus. The gametes fuse to form a zygote,

which subsequently secretes a cyst wall to develop into Oocysts. Oocysts rupture the intestinal epithelial cells to disseminate into the lumen and hundreds of millions are excreted in feces for days or weeks. Oocysts undergo sporulation outside of the body to become infective to other hosts (Dabritz and Conrad, 2010), as show in figure (2-4). Cats with toxoplasmosis typically show no signs of the disease (Elmore *et al.*, 2010).

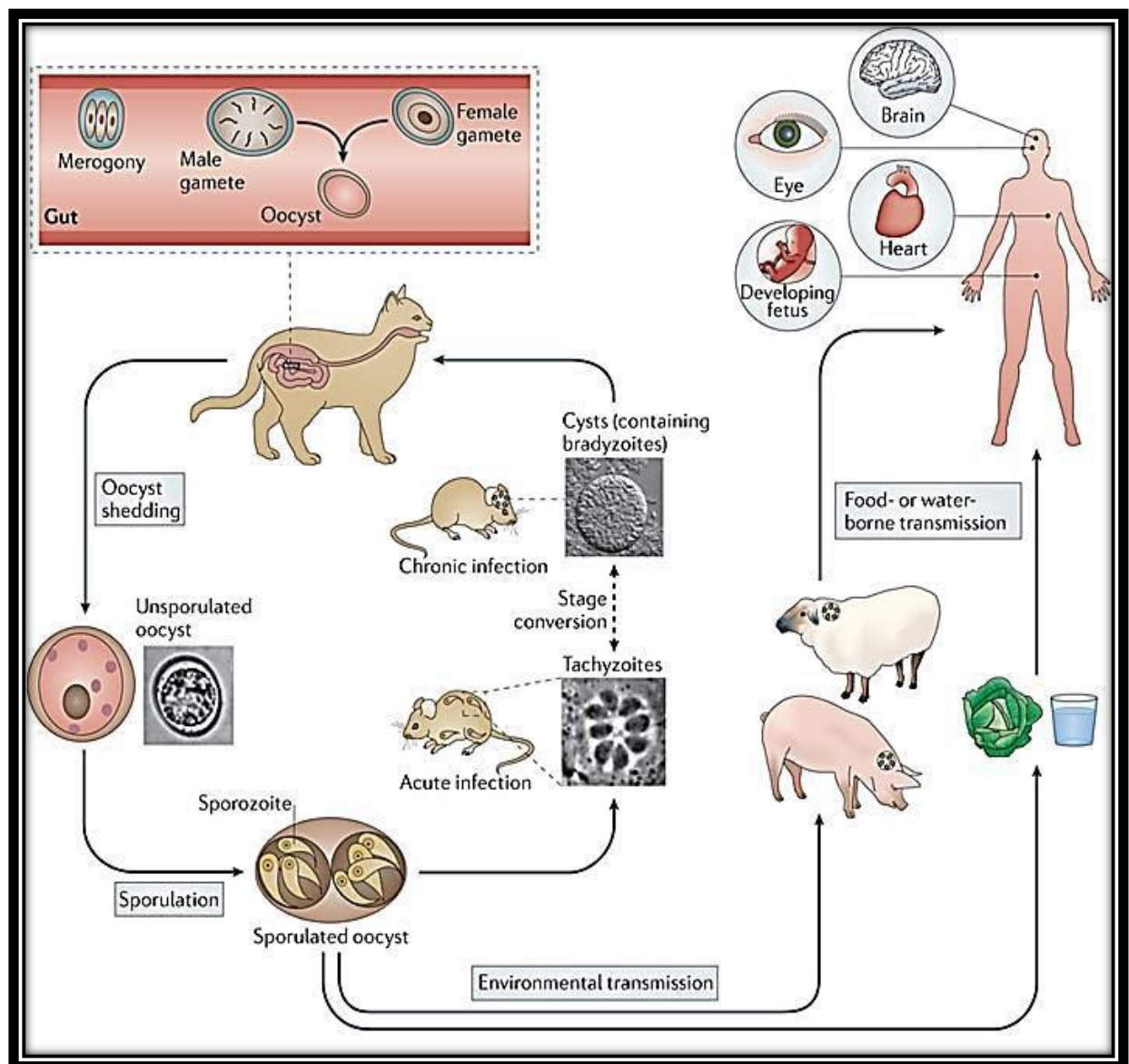


Figure 2-4: life cycle of *T. gondii* (Hunter and Sibley, 2012).

2.4 Classification of *T. gondii* :

T.gondii belongs to the phylum Apicomplexa, class Coccidia and subclass Eucoccidiorida, this classified according to (De Craeye, 2012) as follows:

Kingdom : Animalia

Sub Kingdom: Protozoa

Phylum: Apicomplexa

Class: Coccidia

Subclass:Eucoccidiorida

Order: Eimeriorina

Suborder : Eimeriina

Family: Sarcocystidae

Genus: *Toxoplasma*

Species: *T.gondii*

2. 5 Epidemiology :

There are many factors that play important role for distribution *T.gondii* in the world such as sanitary habits ,ethnic considerations ,low education, age and animal contacts (Gaetano *et al .*, 2010). All factors that description may be enhanced antibodies production against *T.gondii* , also there are other factors are very important like climatic factors ,because it is provide the suitable environments for Oocyst survive in some conditions (Jones,2006). Low seroprevalences 10-30% have been observed in North America, in south East Asia, in northern Europe and in Sahelian countries of Africa. Moderate

prevalences 30 - 50% have been found in countries of Central and Southern Europe, and high prevalences have been found Latin America and tropical African countries (Robert-Gangneux and Darde, 2012). In Latin America human toxoplasmosis is known to be highly endemic, In Mexico, Central and South America and the Caribbean Islands with antibodies detectable in as many as 65% of the population and 46% in Venezuela (Chocin – Bonilla *et al.* ,2001). In Brazil, toxoplasmosis is an important public health problem with serological prevalence ranging from 50-54% in adult (Bahia- Oliverira *et al.*, 2003). In Turkey, serosurvey of Toxoplasmosis among women denoted that 49.4% are positive (Al-Saadii, 2013).

In Arab countries the prevalence rate among people from different Arab countries using different techniques, it was 53% in Somalia ,while in Saudi Arabia were 31.2 % of pregnant women attending Abdul-Aziz University Hospital in Jeddah (AL-Mosawi, 2014). In Amman (Jordan) 47% of pregnant women showed seropositivity to *Toxoplasma* with a statistically significant increase in the rate of seropositivity with increasing age and parity and it was higher among women living in rural areas who are using rainwater to drink, ingesting undercooked meat and have contact with soil (Qublan *et al.*, 2002). Al-Nahari and Al-Tamimi (2010) recorded from 463 pregnant women serum samples from detection of specific anti-*Toxoplasma gondii* antibodies by ELISA at Sana' capital and capital Trusteeship in Yemen ,194 samples 41.90%were seropositive for anti *T.gondii* IgG and 55 (11.88%) for anti *T. gondii* IgM. By using skin test Najim and Al-Safar (1961) observed 20.5% occurrence of toxoplasmosis among general population and they recorded in 1963 infection cases rate 40.5% in women with history of miscarriage by same test(Al-Saadii, 2013). Fatohi, (1985) found the infection rate in recurrent aborted women in Mosul was 40.25% and 33.07% by CFT and IHAT respectively, whereas Tawifiq, (1983) found that the rate of toxoplasmosis

among pregnant women in Baghdad was 30% (AL-Mosawi, 2014). In Kurdistan region (Sulaymania, Arbeel and Duhok), Niazi *et al.* (1992) found that the rate of infection was 15.62% among aborted women. Hasson (2004) recorded that, the *Toxoplasma* infection rate of positive case in Najaf province was 133(40.4%) out of the total number of (325) pregnant women, 64 (19.7%) were positive by LAT and 8.92%, 12.3% were positive by IgM-EIA and IgG-IFAT respectively. Yacoub *et al.* (2006) found that the rate of infection was 59.1% among aborted women in Basra. In a study of Thi-Qar by Al-Addlan (2007) found the rate of *Toxoplasma* infection was 61.62% by using LAT and 32.46%, 18.42% using ELISA IgG, ELISA IgM respectively and by using technical PCR infection rate was 18.67%.

2.6 Transmission of *T. gondii*

There are many important modes for transmission *T.gondii* in human different and based on some factors such as eating habits, social culture and environmental factors (Demar *et al* ,2007) . Human infection may be acquired in several ways:

- 1- By ingestion the food ,water and unsterilized milk that contaminated with Oocyst which released from infectious cats or by consumption undercooked meat that containing the tissue cysts (Sakikawa *et al* ,2012) .
- 2- Congenital transmission from pregnant women to fetus through transplacenta , which developed in certain intermediate host by *T.gondii* for maintain the reservior infection (Hill and Dubey,2002) .
- 3- Human can acquire *T.gondii* from tachyzoites in,blood products and tissue transplants (Tenter *et al.*, 2000).

2.7 Diagnosis of Toxoplasmosis :

The clinical diagnosis of toxoplasmosis is difficult, because it can remain latent asymptomatic for unpredictable length of time, therefore, laboratory tests including serological tests are essential to detect Abs to *T. gondii* (Liesenfeld *et al.* , 2001). Depending on the phase and the strain of *T.gondii* can be detect the acute and chronic infection by serological techniques including Enzyme-linked immunosorbent assay (ELISA) also known as an enzyme immunoassay (EIA), is a biochemical technique used generally in immunology to detect the presence of an antibody (immunoglobulines such as IgG ,IgM ,IgA and IgE) or an antigen in a sample patients (Singh, 2003). In first infection of *T. gondii* IgM arise within a few weeks after exposure to this parasite ,followed by IgA then IgE this status called acute infection, Immunoglobulin's peak after approximately 2 months and are usually cannot detect through serological technique by six to nine months but can persist for longer time (Montoya and Ross , 2005). IgG antibody appears after IgM, peak after 4 months and persist at low level for long time in the body of host (Sukthana,2006). The diagnosis of this parasite usually by many laboratory technique including PCR, Histological detect and immunological testing. Cerebral toxoplasmosis was diagnosed by computerized tomography and magnetic resonance imaging (Sukthana, 2006) . Also there are other methods for *Toxoplasma* infection diagnosis such as modified agglutination test (MAT) , the Sabin – Feldman dye test that is the traditional gold standard, complement fixation test (CFT), indirect fluorescent antibodies assay (IFA) and immunoblotting (Dubey,2010).

2.8 Pathogenicity

The pathogenesis of toxoplasmosis differ depending on host factors like genetic background and immune system as well as the characteristics of parasite such as virulence of the strain (Montoya and Liesenfed , 2004).

The parasite may transport first to the mesenteric lymph nodes and spread to other organs by enter of lymphatic's and blood stream (John and Petri, 2006). When *T.gondii* infects the cells can be produces the formation of a parasitophorous vacuole (PV) (Carruthers and Sibley,1997). Tachyzoites attack the macrophages and dose not trigger oxidative killing mechanisms (Montoya and Rosso , 2005). The tachyzoites forming an intracellular pseudocyst during dividing by binary fission that leads to cellular distruption (Nash *et al* .,2005). Infection in pregnant women, tachyzoites will be spread to the placenta and may result abortion or transmitted transpla-centally directly to the fetus and the results in hydrocephalus, retinochoroiditis and intracranial calcification(Giannoulis *et al* ., 2008). Most signs of toxoplasmosis including lymphadenopathy , which may be supplemented by fever, exhaustion, headache, muscular or abdominal pain also hepatitis, myocarditis and pulmonary necrosis (Markus, 2003). Clinical manifestations of acute retinocoroditis include pain , tearing photophobia and progressive loss of vision over time especially when there is macular or optic nerve involvement (Dubey, 2008) .

2.8.1 Toxoplasmosis in Immunocompromised Patients

In contrasting with the setting of *Toxoplasma* infection in immunecompetent individuals, toxoplasmosis is always life threatening in immunocompromised patients, yet the host immune background is prime importance. Various factors responsible for impaired cellular immunity can lead to severe toxoplasmosis, among which are HIV infection and immunosuppressive therapies. Patients are more commonly a risk for disease reactivation resulting from cyst rupture than for newly acquired infection, but the risk may differ among categories of patients (Montoya and Liesenfeld, 2004).

Toxoplasmic encephalitis (TE) is the predominant manifestation of the disease in these patients and can lead to various symptoms, ranging from headache lethargy, incoordination, or ataxia to hemiparesis, loss of memory, dementia, or focal to major motor seizures and usually associated with fever (Luft and Remington, 1992). Less frequently, toxoplasmic retinochoroiditis may occur independently any other signs of evolutive infection and must be distinguished from other infectious etiologies, in particular eye lesions due cytomegalovirus (CMV), HIV or syphilis (Robert-Gangneux and Darde, 2012). Following transplant, the parasitic infection is clearly related to the type and duration of suppressive therapy (Derouin *et al.*, 2008).

2.8.2 Cardiac toxoplasmosis:

T. gondii is the second relevant pathogen in protozoan myocarditis (Kirchoff *et al.*., 2004). In 2000, Sano *et al.* stated that only 22 cases of toxoplasma pericarditis had been reported worldwide, 15 of them in absence of immunosuppressant disorder. They found that fever, dyspnea and chest pain were the usual symptoms at the onset while seven patients had then developed cardiac tamponade requiring pericardiocentesis (Sano *et al.*, 2000). In spite of such low number of cases of myocarditis by *T. gondii*, many authors agree that toxoplasmosis should be considered in patients with myocarditis, pericarditis and polymyositis of unclear etiology, due to the effectiveness of specific therapies (Mroczeck-Czernecka *et al.*., 2006) . Cardiac toxoplasmosis is a rare disease and literature search described 41 cases of toxoplasmosis of heart in several necropsy series (Lanjewar *et al.*., 1998). The low reporting of toxoplasmic myocarditis may be because these lesions remain localized in the myocardium of the left ventricle, hence extensive tissue sampling of heart is essential to rule out toxoplasmosis (Hofman *et al.*., 1993).

Cardiac toxoplasmosis generally occurs during the course of multivisceral dissemination, however there are 12 reported cases of toxoplasmic myocarditis in which sudden death is described, reactivation of toxoplasmosis is the most common type of toxoplasmosis found in immunocompromised patients. *T. gondii* resides intracellularly in phagosomes within macrophages and myocardial cells (Sahasrabudhe *et al.* , 2003) .

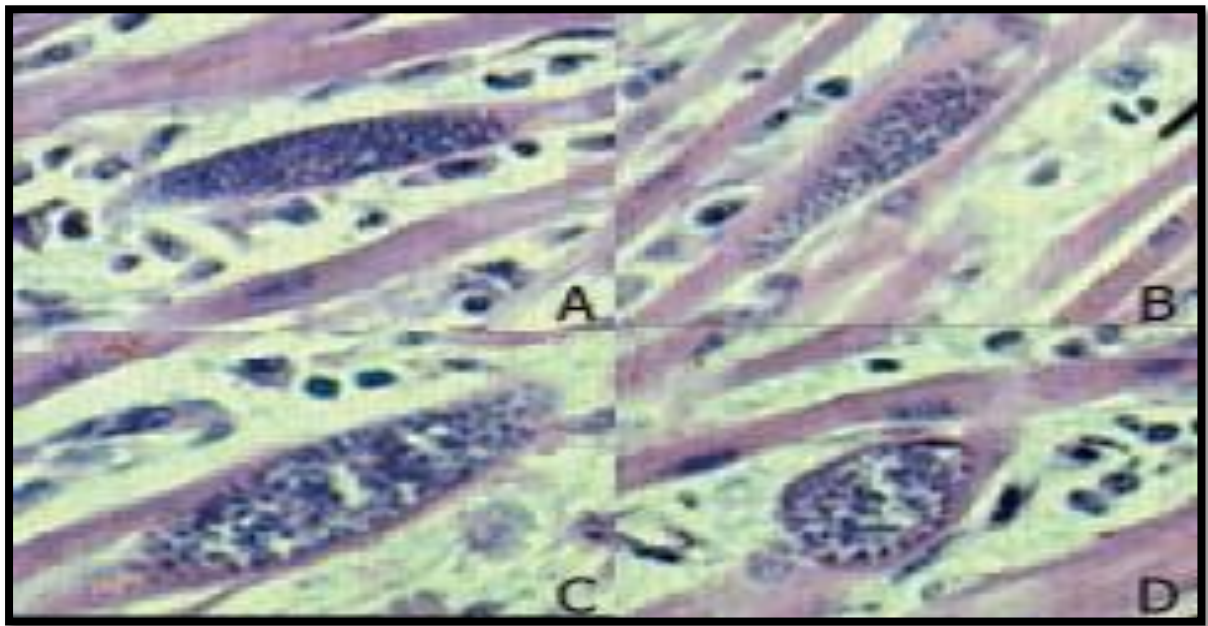


Figure 2- 5: (A& B, H&E x300), and (C& D, H&E x 600): Microscopy shows enlarged myocardial muscle containing pseudocyst of *T. gondii* (Lanjewar *et al.*, 2006).

2.9 Immune response to *Toxoplasma gondii*

Protection against *T. gondii* acquired via oral ingestion of tissue cyst containing bradyzoites or oocyst harbouring sporozoites is provided by numerous non immunological factors, including barriers that are both chemical (pancreatic proteolytic enzyme, gastric acid, mucus, bile) and physical (gut motility, epithelial cell layer) (Buzoni-Gatel and Kasper, 2007).

2.9.1 Innate Immunity in Toxoplasmosis

2.9.1.1 Role of dendritic Cells

The maturation and activation of DCs in response to infection plays a key role in initiating the innate and adaptive immune responses. DC maturation is defined by the up-regulation expression of major histocompatibility complex (MHC) and co-stimulatory molecules(e .g CD 40) that enhance the proliferation of naïve T cells, while activation is defined by the production, of inflammatory a cytokines that regulate T cell differentiation (Gubbles *et at.*, 2005). In response to local infection ,antigen loaded DCs migrate from the site of infection to the spleen and draining lymph nodes and present antigen to naïve T cells. During *T.gondii* infection, the production of IL 12 by DCs baies the CD4 T cell response to parasitic –specific Th1 immune response (Reis e Sosa *et al.*, 1999).

2.9.1.2 Role of Macrophages

Besides the production of pro-inflammatory cytokines such IL12, the major functions of macrophages are to detect, through several different pattern recognition receptor(PRRs) on their cell surface, and eliminate, through phagocytosis, pathogen. To limit the initial dissemination and growth of pathogen, macrophages turn on their microbicidal effector mechanism such as phagolysosomal degradation and production of reactive oxygen intermediates (ROI) and nitric oxide (NO) (Mosser,2003;Han, 2012). Among the important molecules needed for macrophage activation, IFN- γ is produced by NK and T cells whereas TNF α is produced by the macrophages themselves in response to pathogen associated molecular patterns (PAMPS) (Stafford *et al.*,2002). These two signals lead to upregulation of inducible nitric oxide synthase (iNOS) and p47 GTPases (Taylor *et al.*,2007). Activated macrophages are able to eliminate parasites that have invaded the cell. The elimination of invaded parasites inside

the PV is thought to be mediated by the process of autophagy (Ling *et al.*, 2006). Interaction of CD40/CD40L can trigger the IFN γ - independent process of autophagy (Yap *et al.*, 2007). Autophagosomes surround the PV and initiate lysosomal degradation of the parasite. An IFN γ -dependant mechanism for elimination of parasites is the activation of p47 GTPases. IFN γ induced activation the GTPases causes GTPase migration to the PV where they disrupt it and release the parasites from the protective vacuole. The mechanism of this process is still unclear (Taylor *et al.*, 2007).

2.9.1.3 Role of Natural Killer Cells

Natural killer cells are a major source of IFN- γ in the early phase of the immune response to *T.gondii* infection . IL12 initiates NK cell killing of infected cells (Hunter *et al.*, 1994 b). NK cells develop in the bone marrow and circulate in the blood. After infection, NK cells migrate to the lymph node and then to the site of infection where they release IFN- γ to stimulate activated macrophages increasing cell surface MHC II class expression NK cells express a variety of chemokine (Combe *et al.*, 2006).

2.9.2 Adaptive Immunity in Toxoplasmosis

2.9.2.1 Cellular Immune Response

2.9.2.1.1 Role of T Cells

The effector cells are stimulated by dendritic cells presenting the antigen to T-cell receptor. However, this mechanism requires a close interaction between the antigen presenting cells (APCs) and the T-Lymphocyte (TL) thanks to the CD40- CD40L system (Reichmann *et al.*, 2000). Infection of the dendritic cells by live *Toxoplasma* exclusively leads to activation of CD40 in

human (Subauste and Wessendarp, 2000). These effector cells, which are involved in resistance to *Toxoplasma* infection, then exert their function via cytotoxic activity and secretion of cytokines involved in the regulation of immune response (Hunter *et al.*, 1994 a). T cells are recruited by different chemo attractants to the site infection. The cytokine INF- γ promotes the differentiation toward Th1 cell through the recognition of antigen, presented by (APCs), by the precursor cells, the differentiation and proliferation to Th1 cells is initiated and cytokines such as IL2 and additional INF- γ are produced (Villegas *et al.*, 2002). Further, the release of IL2 in the system triggers the activation and proliferation of antigen-specific CD8⁺ effector T cells. These antigen –specific CD8⁺ effector T cell have cytotoxic activity, which leads to killing of infected cells and more INF- γ production. Exactly, the activation of T cells creates a feedback loop that induces the production of more INF- γ . The persistence of the memory of T-lymphocyte in toxoplasmosis in human is an established fact (Gigley *et al.*, 2011).

2.9.2.1.2 Role of B Cells

By contrast with T cells, B cells are less prone to circulate, but rather move within secondary lymphoid organs until they are activated and transform into an antibody secreting plasma cell or memory cell. They subsequently relocate to other lymphoid organs or the bone marrow (Friedl and Weigelin, 2008).

2.9.2.2 Humeral Immune Response in Toxoplasmosis

Humoral immune response varies with the stage of infection, hence some antibodies present at one stage of infection may be absent in other stages and vice versa. This requires multiple epitopes from different antigens be present in an immunoassay to detect the antibodies present in the different disease states (Pelloux *et al.*, 2006). High level of antibodies IgM, IgA and IgG directed against *Toxoplasma* antigens (Roitt *et al.*, 2001), IgE elevation was not observed in patient with toxoplasmosis (Jones, 2006).

IgM and IgA levels rise in parallel in the acute phase of the infection, later IgG appears. After 2-8 months, IgM and IgA levels usually drop and disappear (Correa *et al.*, 2007). Some time IgM antibodies persist for over a year (Flori *et al.*, 2008). Extracellular tachyzoites are lysed by specific antibody when it is combined with complement (Mahalakshmi, 2006). Tachyzoites exposed to antibodies were no longer capable to enter various cells and were readily engulfed by macrophages (Nash *et al.*, 2005).

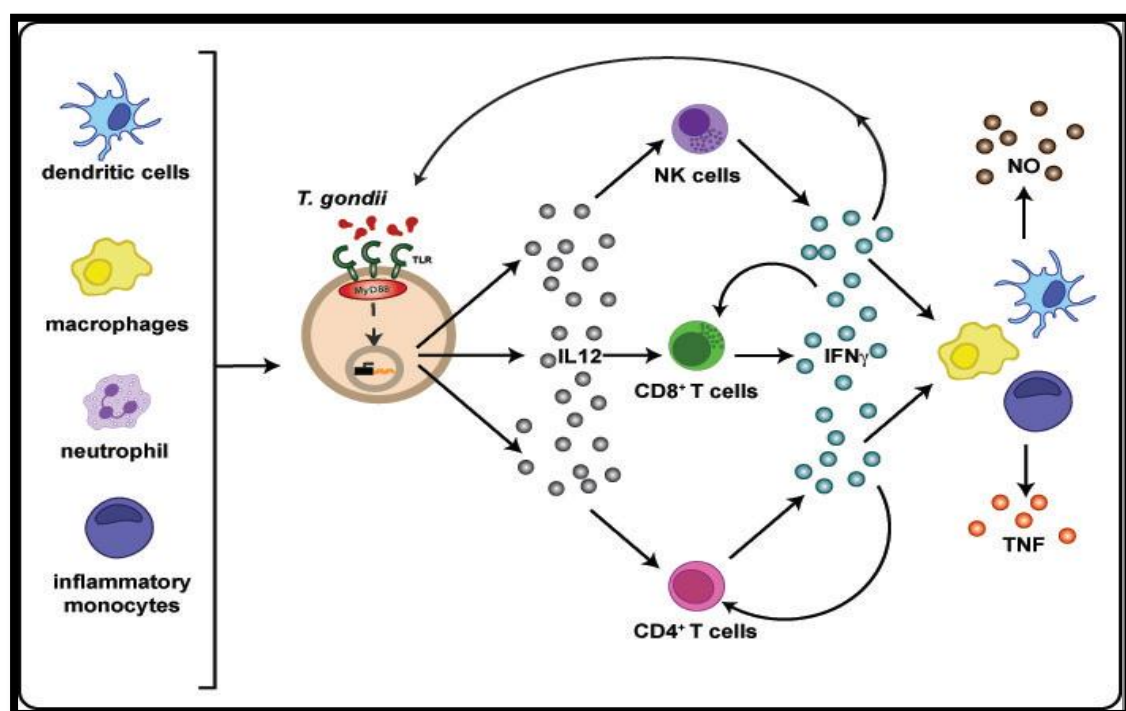


Figure 2-6: Initiation of the acute immune response to *Toxoplasma gondii* (Han, 2012)

2.10 Cytokines

Cytokines have been described as a vast array of relatively low molecular weight, pharmacologically active proteins that are secreted by one cell for the purpose of altering either its own functions (autocrine effect) or those of adjacent cells (paracrine effect) (McDermott, 2001). In many instances, individual cytokines have multiple biological activities. Different cytokines can

have the same activity, which provides for biological redundancy within the inflammatory and immune systems. (Patrizi ., 2008) .

Cytokines are produced by various immune system cells and perform several functions, including mediation of the immune and inflammatory responses. The effects of cytokines on the immune response depend on a number of factors, such as their local concentrations, receptor expression patterns and the integration of multiple signalling pathways in response to immune cells (Gaffen , 2004) . Cytokines are key signalling molecules in inflammation, exerting a regulatory effect in both the innate and the adaptive immunological response. Cytokines bind to either specific cellular receptors or soluble receptors capable of modulating the immunological effect of cytokines, The immune response is further moderated by cytokine receptor antagonists, also binding to cytokine receptors (Drexhage *et al.*, 2010a). Importantly, the role of cytokines in metabolism extend beyond the inflammatory system, impacting also on neurotransmitter metabolism, neurogenesis and the neuroendocrine system (Haroon *et al.*, 2012). Cytokines are protein messengers that convey information between and within the immune system via specific cell surface receptor molecules. Traditionally, Cytokines can be divided into pro-inflammatory cytokines and anti-inflammatory cytokines, based on global effects in animal models or direct effects on individual immune-cell populations (Stegall , 2010). Interleukins are small protein molecules that signal specific cells to regulate the immune systems of organisms, they are primarily synthesized by T cells, monocytes, macrophages and endothelial cells (Gomes ,2016). The functions of ILs include the facilitation of communication among immune system cells, regulation of transcription factors, and control of inflammation, cell differentiation, proliferation and antibody secretion (Salazar-Onfray *et al* ., 2007).

Cytokines such as IFN – γ and α (which activate macrophages function) are important for controlling tachyzoite replication during both acute and chronic phases of infection, IL-12 is clearly important in initiating a strong and effective cell-mediated immunity against *T. gondii* tachyzoite, IL-10 appears to modulate both IL-12 and IFN – γ synthesis *in vivo*, avoiding an excessive immune response that could cause extensive inflammation and host tissue damage. IL-10 and IL- 12 are two major antagonist involved in regulating IFN- γ synthesis during the initial phases of infection. (Butcher *et. al*, 2005) . This simple dichotomy might not fully describe the pleiotropic role that several cytokines, such as interleukin- 10 (IL-10), have during the early immune response. Indeed, the function of a given cytokine is determined by its tissue levels, the nature of the target cell and activating signal, the timing and sequence of cytokine exposure and, more generally, the experimental model tested, as discussed for a wide range of cytokines (Cavaillon , 2001).

2.10.1 Interleukin 12

Interleukin-12 was independently discovered by Trinchieri and colleagues (in 1989) also by Gately and colleagues (in 1990) as “natural killer–stimulating factor” and as “cytotoxic lymphocyte maturation factor” respectively, interleukin 12 (IL-12) is a disulphide-bonded, it was identified as a heterodimeric cytokine composed of two covalently linked p35 and p40 subunits each of which is expressed on different chromosomes (Stern *et al* .,1990). It is involved in the stimulation and maintenance of Th1 cellular immune responses, including the normal host defence against various intracellular pathogens, such as *T. gondii* . IL-12 also has an important role in enhancing the cytotoxic function of NK cells (Abbas *et al* ., 2012) and role in pathological Th1 responses, such as in inflammatory bowel disease and multiple sclerosis. Suppression of IL-12 activity in such diseases may have therapeutic benefit. On the other hand, administration of recombinant IL-12

may have therapeutic benefit in conditions associated with pathological Th2 responses (Park, 2001). The general model on the biological role of IL-12 predicts that this cytokine is required for resistance to bacterial and intracellular parasites, as well as for the establishment of organ-specific autoimmunity (Trinchieri, 2003). According to such a model, IL-12, produced by activated phagocytic cells (monocytes, macrophages and neutrophils), by dendritic cells (DC), and by the identified IFN-producing killer DC lineage (Chan *et al*., 2006), which acts as a critical regulator of cell-mediated responses.

The biological functions of IL-12 are mediated by the IL-12 receptor composed of two chains (h1 and h2) (Trinchieri *et al*., 2003). In *T. gondii* infection caused by oral route, IL-12 acts synergistically with IL-18 to produce IFN- γ by NK cells, as shown in figure (2-7). IFN- γ synergizes with IL-12 to drive the differentiation of Thp to Th1 phenotype, express IL-12 receptor on T cells, and inhibit the antagonist IL-4 to prevent the differentiation of Thp towards Th2 phenotype (Denkers and Gazzinelli, 1998).

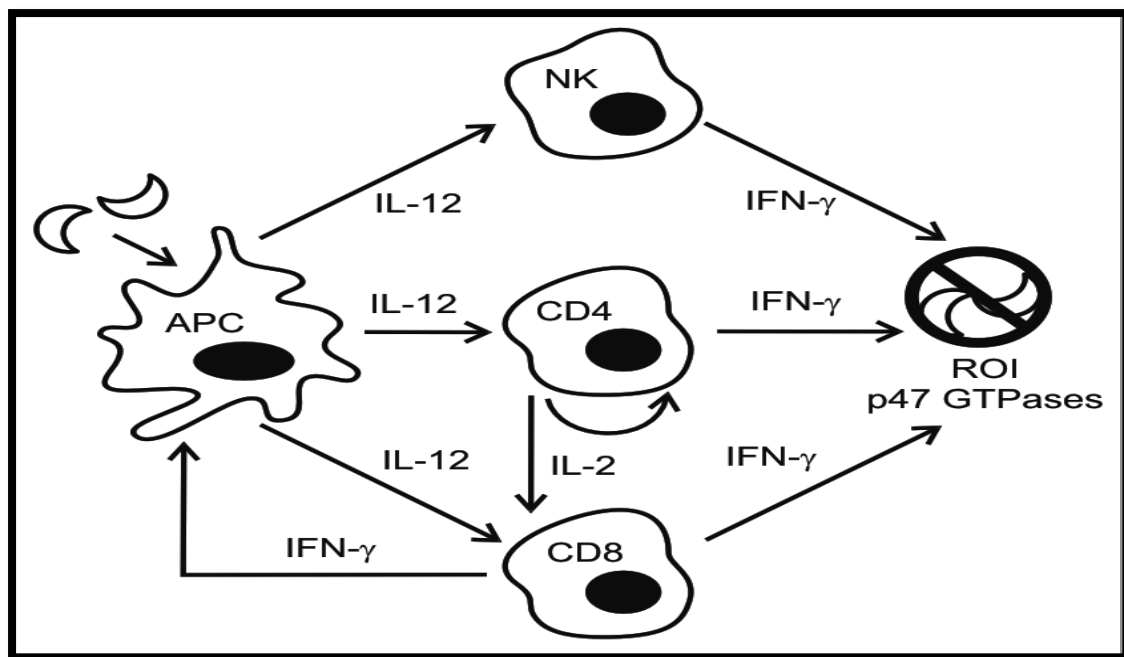


Figure 2-7: The role of IL-12 in toxoplasmosis (Tait and Hunter, 2009)

2.10.2 Interleukin-10

In 1989, a new cytokine, originally called cytokine synthesis inhibitory factor, and later nominated as IL-10, was identified based on its skill to antagonize T helper 1 (Th1) responses (Fiorentino *et al.*, 1989). Currently, IL 10 is considered to be an inhibitor of Th1, Th2 and Th17 immune responses (O'Garra, 2007). IL-10 is a protein that also inhibits the synthesis of a number of cytokines, including IFN- γ , IL-2, IL-3, IL-12, IL-6, microglia in the brain, and Granulocyte Macrophage - Stimulating Factor (GM-CSF) produced by activated macrophages and by helper T cells. In structure, IL-10 is a protein of about 160 amino acids that contains four conserved cysteines involved in disulphide bonds (Zdanov *et al.*, 1995). Interleukin 10 is encoded by the IL10 gene (Eskdale *et al.*, 1997). IL-10 is a cytokine with anti-inflammatory properties including counteracting the function of Th1 lymphocytes. IL-10 plays a vital role in controlling the inflammatory response during chronic phase of *T. gondii* infection (Wilson *et al.*, 2005). IL-10 (18 kD) is a product of immunoactive cells, including B cells, macrophages, DCs, Th2 cells, T-regulatory cells and CD4⁺T cells; that has been regarded as one of the most important anti-inflammatory immune-regulating cytokines (Levings *et al.*, 2002). presumed to keep homeostatic network and protect tissue from collateral damage caused by excessive inflammation (Owen *et al.*, 2013). IL-10 signals through a receptor complex consisting of two IL-10 receptor-1 and two IL-10 receptor 2 proteins (Mosser, 2008).

IL-10-deficient mice showed elevated IL-12 levels and consequently increased IFN- γ and TNF- α responses and intense hepatic inflammation and tissue necrosis (Gazzinelli *et al.*, 1996). The recent interest in IL-10 as a potentially protective cytokine in the atherosclerotic process is based on the hypothesis that the development of plaque-forming atherosclerosis is a localized inflammatory process of the vascular wall (Ross, 1999). Indeed, IL-10

may act as an antiatherogenic factor by a number of different mechanisms apart from mere inhibition of pro-inflammatory cytokines. First, IL-10 interferes with the first step in atherogenesis by inhibiting attachment of circulating immune cells to the endothelium through down-regulation of the adhesion molecules CD18, CD60L, and intercellular adhesion molecule-1 (ICAM-1) (Song *et al.*, 1997). Second, IL-10 inhibits the secretion of chemotactic proteins by macrophages that might attract further leukocytes to the location of subendothelial inflammation(Olszyna *et al*, 2000) Moreover, IL-10 reduces the production of lytic enzymes produced by monocytes, such as matrix-metalloproteinases (enzymes associated with destabilization of a plaque), and suppresses superoxide anion production(Kuga *et al.* ,1996), suggesting that IL-10 could prevent plaque destabilization (Lacraz *et al.* , 1995).

2.10.3 Interleukin-17

The Th1–Th2 paradigm has dominated the study of immune- mediated resistance to infection for almost 20 years, with the initial description of IL-17 as a cytokine produced by T cells (Harrington *et al* 2006). Interleukin 17-A or IL-17 identified by Rouvier *et al*, (1993). It was not clear how this pro-inflammatory factor related to the biology of established Th subsets (Weaver *et al* 2006) . Early reports identified a role for IL-17 in the development and recruitment of neutrophils to sites of inflammation (Witowski *et al* , 2004), but studies by Cua and colleagues highlighted the ability of IL-17– producing CD4+ T cells, termed Th17 cells, to contribute to the development of inflammation in models of rheumatoid arthritis and experimental allergic encephalomyelitis (Langrish *et al* ,2005) ; It is now appreciated that there are additional cellular sources of IL-17 that contribute to a variety of inflammatory conditions, including CD8+ T cells (Intlekofer *et al* 2008) , and more recently invariant NKT cells (iNKTs) (Rachitskaya *et al* 2008).

The IL-17 family of cytokines consists of six family members of varying homology and function: IL-17 (also called IL-17A), IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F. In general, these cytokines range from 20–30 kDA and incorporate four conserved C-terminal cysteine residues, the cytokines IL-17 and IL-17F share the strongest homology, which is likely the reason why these mediators are thought to use similar signaling mechanisms in exerting their pro-inflammatory functions(Chang and Dong ,2009). The cytokines IL-17 and IL-17F have been associated with a distinct lineage of CD4⁺ T helper lymphocytes (Th) known as Th17 cells (Weaver *et al.* , 2006).

IL-17 has been extensively studied since its discovery in human peripheral blood (Aarvak *et al.* ,1999). The identification of IL-17 led to the unearthing of the specialized Th17 subset that was found to produce the cytokines IL-17 as well IL-17F (Dong , 2008). IL-17 is a potent proinflammatory cytokine produced by activated memory T cells (Aggarwal and Gurney , 2002). The development of a protective host response to intracellular pathogens requires the coordinated action of the innate and adaptive immune system. For many pathogens, the interaction between DCs, macrophages, NK cells and neutrophils provide a limited mechanism of innate resistance during the early stages of infection, and also influence the development of adaptive immunity required for long-term protection (Denkers *et al.*, 2004). Produce of IL-17 derived from innate and adaptive sources of immune response. Specialized T cells, called Th17 cells, are the major source of IL-17 in many types of adaptive immunity. There are several types of innate immune cells that also produce IL-17. These cells are localized to mucosal tissues, such as the intestine, skin and lung, which constantly interact with the outside environment and can respond via their expression of Toll-like receptors(Chien and Bonneville, 2006). In toxoplasmosis the role of IL-17 was involved in the development and early recruitment of neutrophils, which are essential to clear the parasites during

initial stages of infection. The initial of innate immune response by neutrophils has also been reported to be critical for successful (Yisong and Richard, 2009).

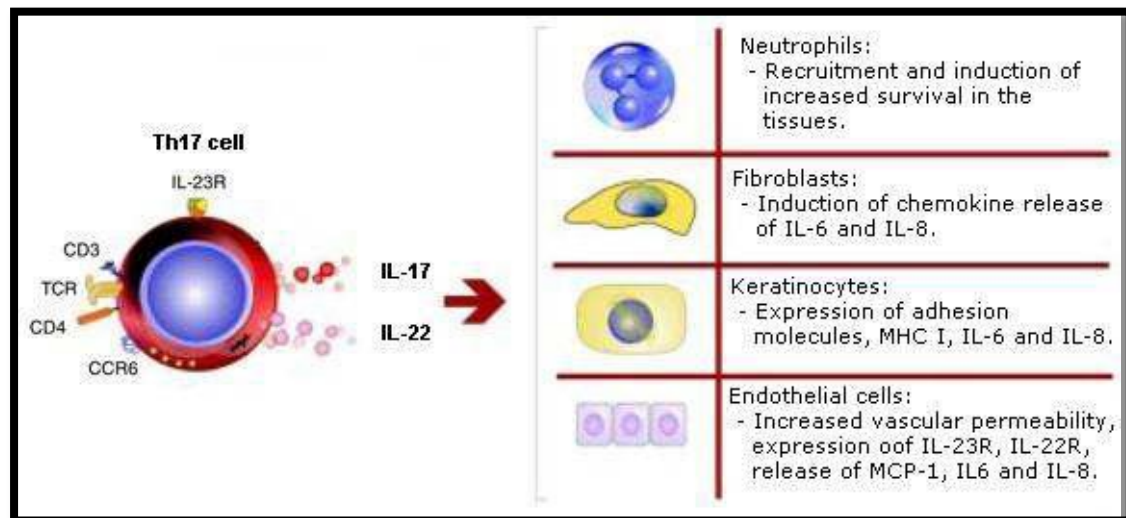


Figure 2-8: Effect of Th17 cells on several cell types Production of IL-17 and IL-22 (Mesquita *et al*, 2009).

2.11 Heart Disease

It is a global serious health problem especially in people aged above 65 years (Sanderson and Tse, 2003). It is estimated that nearly 6.5 million people in Europe, five million people in the USA, and 2.4 million people in Japan suffer from heart disease. In developing countries its incidence is steadily increasing (Barker *et al* ., 2006). Heart disease is a broad clinical syndrome with many different underlying etiologies. In some studies ischemic heart disease (IHD) was the commonest cause and in other studies arterial hypertension (AHT) was the most common (Lip *et al.*, 2000 ; Fox *et al.*, 2001). In developing countries bacterial infection as streptococcal infection and its role in the etiology of rheumatic heart disease play an important role (Rich , 1997). Multiple pathogens have been implicated in the pathogenesis of myocarditis, including *Toxoplasma gondii*, *Mycobacterium tuberculosis*, *Cryptococcus*, *Cytomegalovirus*, *Herpesvirus*, HIV and *Trypanosoma* (Kaul *et al.*, 1991).

Trypanosoma cruzi, the causative agent of Chagas' disease, approximately 18 million people are infected, the majority residing in the endemic areas of Central and South America. Ten to thirty per cent of infected individuals develop clinical disease associated with congestive heart failure or mega syndromes of various hollow organs (e.g. oesophagus, colon or ureter). Numerous reports of severe forms of Chagas' disease exist in the immunocompromised patient, these most frequently include meningo-encephalitis and acute myocarditis, individuals with lymphoreticular neoplasias receiving immunosuppressive therapies, as well as renal, bone marrow, and cardiac transplant patients appear to be at greatest risk for severe clinical manifestations (Ferreira and Borges 2002). These disease manifestations typically result from the reactivation of chronic infections, although acute forms resulting from blood transfusions have also been reported, experimental models of chronic infection with *T. cruzi* demonstrate reactivation of the disease with an increase in parasitaemia, exacerbation of myocarditis and myositis, as well as increased mortality in mice submitted to a number of different immunosuppressive regimens (Andrade *et al.*, 1997).

Recently, evidence-based pharmacological and device therapies indicated the decrease in heart failure symptoms, hospitalization, mortality and improve quality of life of patients with heart disease, still many patients treated with these regimens often remain burdened by dyspnea and fatigue, diminished exercise tolerance, reduced quality of life, recurrent hospitalizations and early mortality, and if underlying cause is not corrected, more than half of the patients with heart disease will die within 1-4 years depending on the severity of the disease (Fonarow *et al.*, 2005).

2.12 Prevenation and Treatment

There are some effective drugs are widely used for treatment of toxoplasmosis (Guerina *et al* ., 1994). Pyrimethamine (Daraprim) and Sulphadiazine are very important to confirm the infection of the toxoplasmosis (Jennifer *et al* ., 2003). Pyrimethmine is widely used for treatment the infection *T.gondii* , this drugs play role by blocking the metabolic pathway involving folic acid and P-aminobenzoic acid cycle respectively (Petersen and Schmidt, 2003). Using vaccine preventes infected cats with *T.gondii* from shedding oocyst (Mateus-Pinilla *et al.*, 1999). All meats of any animal should be cooked to minimum temperature of 67 °C before consumption , so as to kill tissue cyst and tasting meat while cooking should be avoided. Tissue cyst can also be killed by cooling to -13°C. Pregnant women especially should be careful and avoid contact with cats, raw meat and soil (Hill and Dubey, 2002). Hand washing by soap is very effective to kill the stages of *T. gondii* (Dubey and Beattie 1988). Also should washes sink tops , knives, cutting boards and other materials which contact with uncooked meat to prevent infection by *T. gondii* (Lopez *et al* ., 2000). *T. gondii* in tissue cysts are killed by exposure to 0.5 kilorads of gamma irradiation (Dubey and Thayer 1994).

Chapter

Three

Materials and Methods

3. Materials and Methods:

3.1 Materials

3.1.1 Instruments

Instruments used in the present study are showed in table (3-1).

Table (3-1): The instruments used in the present study

Instrument	Company	Origin
Incubator	Memmert	Germany
ELISA reader	Bio Tek	USA
ELISA washer	Bio Tek	USA
ELISA Printer	Bio Tek	USA
Deep freeze	Daewoo	Korea
Timer	Organon	Belgium
Cooling box	ENG GUAN	China
Centrifuge	Kokusan	Japan
Refrigerator	Kokusan	Japan
Haematological autoanalyser	Abbott diagnostic	USA

3.1.2 Apparatus:

The apparatus used in the present study were showed in table (3-2)

Table (3-2): The apparatus used in this study.

Equipments	Company	Origin
Eppendorffs tubes	Afma	Germany
Micropipettes	Eppendorff	Germany
Pipette tips	Sterillin	U.K.
Syringe	Medical ject	Syria

Equipments	Company	Origin
Surgical gloves	Blossom	U.S.A
Rack	Diagnostic	U.K.
Tourniquet	Medical ject	Syria
Plain tubes 10 ml	AFCO-DISPO	Jordan

3.1.3 Laboratory kits:

Different laboratory kits used in this study listed in table (3-3).

Table (3-3): Laboratory kits used in this study :

Kit	Company	Origin
<i>Toxoplasma</i> Ab. (IgG)Enzyme linked Immunoassay Test Kit	Foresight	USA
<i>Toxoplasma</i> Ab. (IgM)Enzyme linked Immunoassay Test Kit	Foresight	USA
Anti-A , Anti-B(ABO blood grouping)	Atlas-Medical	U.K.
Human IL-12 (Interleukin 12) ELISA Kit	Elabscience	China
Human IL-10 (Interleukin 10) ELISA Kit	Elabscience	China
Human IL-17 (Interleukin 17) ELISA Kit	Elabscience	China

3.2 Methods:

3.2.1 Sample Collection :

One hundred fifty samples of blood had been collected at period from August 2016 till November 2016 from patients with heart disease, in Heart Medical Centre of Thi-qar province . According to gender, the patients divide in to (81 male and 69 female) with age ranging 18 and 82 years. One hundred samples of blood had been collected from apparently

heathly persons as negative control ,dividing according to gender in to (55 males and 45 females) with age ranging 19-56. Six millilitre of blood were collected from each person by using disposable syringes, and then the blood was placed in gel tubes and allowed to clot at room temperature, then centrifuged at 3000 round per minute (rpm) for 10 minutes and sera were dispensed into 4 eppendrof-tubes, and stored at - 20 ° C until further analysis.

3.2.2 Blood groups –Anti A,B slide test

Monoclonal ABO blood grouping reagent for slide based on agglutination method (Issitt ,1985).

3.2.3 Detection of anti- *Toxoplasma gondii* antibody (IgG)

The Foresight *Toxoplasma* IgG and IgM ELISA (Foresight, USA) kit was used .The *Toxoplasma* IgG ELISA is intended to evaluate a patient's serologic status to *T.gondii* infection

3.2.3.1 Principle:

Purified *T.gondii* antigen (Ag) is coated on the surface of micro wells. Diluted patient serum was added to the wells, and the *T.gondii* IgG-specific Ab, if present, will bind to the Ag. All unbound materials were washed away. Horse radish peroxidase (HRP) conjugate is added, which binds to the Ab-Ag complex. Excess HRP-conjugate is washed off and solution of tetra methyl benzidine (TMP) reagent was added. The enzyme conjugated catalytic reaction is stopped at a specific time. The color intensity generated is proportional to the amount of IgG –specific Ab in the sample. The results were read by ELISA reader.

3.2.3.2 Reagents:

- Micro titer wells: purified *T. gondii* Ag-coated wells (12x8 wells).
- Sample diluents: 1 bottle (22ml).
- Enzyme conjugated reagent 1 vial (12 ml).
- Negative control: range stated on label. (150µL/vial).
- Positive calibrator: 100 IU/ml (150µL/vial).
- Cut-off calibrator: 32 IU/ml, (150µL/vial).
- TMB reagent (one-step): 1 vial (11 ml).
- Positive control: range stated on label, (150µL/vial).
- Wash buffer concentrate (20x): 1 bottle (50 ml).
- Stop solution: (1N HCL), 1 vial (11 ml).

3.2.3.3 Assay procedure:

- 1- Before start the work, the plates were opened and left for (5 min) at RT to equilibrate there and select the required number of strips for the run and we left reagents and specimens to reach RT (15-30° c) prior testing .
- 2- Prepared (1:25 dilution) of wash buffer . The working wash buffer is stable for 2 weeks at 15-30° c .
- 3- Dispensed (100 µl) of calibrators and controls into the appropriate wells(B1,C1,D1,E1,F1,G1,H1 and A2) also dispensed (100 µl) sample diluents to assigned wells starting at B2 (green reagent) . For the reagent blank, was dispensed in 1A well position. Dispensed 5 µl of specimen to assigned wells starting at B2. Tap the holder to remove air bubbles from the liquid , mix well and covered by sealer .
- 4- Incubated at (37°C) for (30 ± 2) minutes.
- 5- At the end of incubation period the liquid from all wells was removed , the microtiter plate was rinsed and flicked five times with 350 µl of diluted wash buffer.

- 6- Dispensed (100 μ l) of enzyme conjugated to each well, and mixed gently for (10 sec) and covered with sealer.
- 7- Incubated at (37°C) for (30 $\pm$ 2 min.).
- 8- Microtiter plate was washed five times with 350 μ l of diluted wash buffer.
- 9-Dispensed 50 μ l of substrate A and substrate B to each well and mixed gently , then the color changed to blue in wells containing positive samples .
- 10- The microtiter plate was incubated at (37 °C) for (10 $\pm$ 2) minutes.
- 11- About (50 μ l) of sulfuric acid was dispensed into each well, and mixed gently for (10 sec), to stop reaction, this well change the color in wells from blue to yellow.
- 12-At (450 / 630-700 nm) the O.D. was read within (30 minutes) with micro well, reader .

3.2.4 Detection of anti-*Toxoplasma gondii* antibody (IgM)

The Foresight *Toxoplasma* IgM ELISA (Foresight) kit was used .The *Toxoplasma* IgM ELISA is intended for using in detection of IgM status to *T.gondii* in human serum.

3.2.4.1 Principle:

The same principle, reagents, assay procedure of ELISA-IgG technique was adopted in ELISA-IgM detection with few exception, the specific antibody IgM was used instead of IgG specific antibody.

3.2.4.2 Calculation of results:-

Calculation of results for both IgG and IgM antibodies was done in the same manner.

1-The mean of duplicate cut-off calibrate was calculated which represents the cut-off value.

2- The mean of duplicate positive control, negative control and patient sample were calculated.

3-The mean value of each sample (absorbance) was divided by the cut-off value ,and then the results were compared with the following ratios to differentiate between positive ,negative and equivocal samples.

Negative : (ratio absorbance / cut-off < 0.9)

Positive :(ratio absorbance / cut-off $\geq$ 1.0)

Equivocal: ratio absorbance /cut-off $\geq$ 0.9 < 1.0 (sample was retested).

3.2.5 Immunological parameters

3.2.5.1 Estimation of serum Interleukin (IL-12, IL10 and IL-17) levels by ELISA technique.

The Elabscience Interleukin (IL-12, IL-10 and IL-17) Enzyme Immunoassay Test kit (E-EL-H0150) was used. The quantitative determination of total Interleukin (IL-12, IL-10 and IL-17) concentration in human sera by microplate Enzyme Immunoassay.

3.2.5.1.1 Principle:

The ELISA kit uses Sandwich-ELISA as the method . The micro ELISA plate provided in the kit has been pre-coated with an antibody specific to (IL-12, IL-10 and IL-17) . Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody . Then a biotinylated detection antibody specific for (IL-12, IL-

10 and IL-17) and Avidin –Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively and incubated . Free components are washed away . The substrate solution is added to each well . Only those well that contain interleukin , biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color . The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow . The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm $\pm$ 2nm . The OD value is proportional to the concentration of (IL-12,IL-10 and IL-17). Calculated the concentration of IL-12 in the samples by comparing the OD of the samples to the standard curve .

3.2.5.1.2 Kit Components:

- 1- Micro ELISA plate pre-coated with an antibody specific to IL-12,IL10 and IL-17 (8 wells $\times$ 12 strips) .
- 2- Reference standard 2 vials
- 3- Reference standard & Sample diluent 1 vial $\times$ 20 ml
- 4- Concentrated Biotinylated detection Ab 1 vial $\times$ 120 μ l .
- 5- Biotinylated detection Ab diluent 1 vial $\times$ 10 ml .
- 6- Concentrated HRP conjugate 1 vial $\times$ 120 μ l .
- 7- HRP conjugate diluent 1 vial $\times$ 10 ml .
- 8- Concentrated wash buffer (25x) 1 vial $\times$ 30 ml .
- 9- Substrat reagent 1 vial $\times$ 10 ml .
- 10- Stop solution 1 vial $\times$ 10 ml .
- 11- plate sealer 5 pieces .

3.2.5.1. 3 Assay procedure:

1. Before starting the work, the plates were opened and left for 5 min. at room temperature to equilibrate their and select the required number of strip for the run.
2. Reagent preparation :
 - A- Prepared (1:25 dilution) of wash buffer .Diluted 30 ml of concentrated wash buffer into 750 ml of wash buffer . The working wash buffer is stable for 2 weeks at 15-30° c .
 - B- Prepared standard within 15 minutes before used.
 - C- Prepared (1:100 dilution) of Concentrated Biotinylated detection Ab .
 - D- Prepared (1:100 dilution) of Concentrated HRP Conjugate .
- 3- One hundred µl of standard , blank or sample were added to each well , then mixed gently and covered with adhesive sealer.
- 4- The microtiter plate was incubated at 37 ° c for 90 minutes .
- 5- About 100 µl of biotinylated detection Ab was dispensed to each well mixed well and covered by adhesive seal after removed the liquid from the wells .
- 6- Incubated at 37° c for 60 minutes .
- 7- The microtiter plate washed by washing solution three times with 350 µl of wash buffer .
- 8- Dispenesid 100 µl of HRP conjugate to all wells and covered with adhasive sealer .
- 9- The microtiter plate was incubated at 37 ° c for 30 minutes .
- 10- The microtiter plate washed by washing solution five times with 350 µl of wash buffer .
- 11- About 90 µl of substrate solution was added to each well and covered with adhesive seal .
- 12- The microtiter plate was incubated at 37 ° c for 15 minutes .

- 13- Dispensed 50 μ l of stop solution were added to all wells .
- 14- The absorbance in each well was read at 450nm in a micro plate reader.

3.2.5.1.4 Calculation of results

Average of the duplicate readings for each standard and samples, then subtracted the average zero standard optical density (OD) . Created a standard curve by plotting the mean OD value for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.

3.2.6. Blood Parameters

We put 1 ml of blood in EDTA tube and shake it quietly, to determine complete blood picture by haematological auto-analyser, the device pulled 200 μ l of blood consequently the blood was analyzed then the data were processed by the computer. What was used in this study were WBC general count, basophils, lymphocytes, monocytes, eosinophil's, neutrophils and haemoglobin estimation.

3.3 Statistical Methods

The significances of differences in proportions analysed by Fisher's exact test . Data were entered into SPSS version 20 and P value equals or less 0.05 considered statistically significant . Chi-square (X^2) was used to compare between discrete independent variables distribution in present studied groups. T test was used to exam the significance of association between two continuous variables which were obtainable as means and standard deviation.

Chapter

Four

Results

4. Results :

4.1 Dividing of study groups

The results of current study showed presence of anti-*Toxoplasma* Abs(IgG and IgM) in both subjects in 66 of 150 (44%) of heart disease patients, while in apparently healthy controls were 19 of 100 (19%). However, the current results indicate to the presence of significant differences between them in the seropositivity of anti-*Toxoplasma* Abs at $p \leq 0.05$, as summarised in table (4-1).

Table 4-1: Seroprevalence of toxoplasmosis in study groups

Number of samples	ELISA test				Total
	Anti- <i>Toxoplasma</i> positive		Anti- <i>Toxoplasma</i> negative		
	Number	%	Number	%	
Heart disease patients(150)	66	44	84	56	150
Control (100)	19	19	81	81	100
Total	85	34	165	66	
Statistical analysis	X ² =16.711, Df=1, P≤0.05				

4.2 Distribution of study groups according to anti-*Toxoplasma* Abs in serum

The results showed presence of anti-*Toxoplasma* IgG Abs in 65 of 150 (43.33%), and 1 IgM of 150 (0.66 %) of heart disease patients. The results also showed presence of anti- *Toxoplasma* IgG Abs in 17 of 100 (17%) in apparently healthy group, and presence of anti-*Toxoplasma* IgM Abs in 2 of 100 (2%). However, the current results indicate to the presence of significant differences between heart disease patients and controls in the seropositivity of

anti-*Toxoplasma* Abs (IgG) at $p \leq 0.05$, whereas no significant difference between both groups in the IgM results at $p \leq 0.05$, in table (4-2):

Table 4-2: Seroprevalence of anti-*Toxoplasma* Abs in heart disease patients and apparently healthy controls using ELISA

Number of samples	ELISA test							
	IgG Positive		IgG Negative		IgM Positive		IgM Negative	
	N	%	N	%	N	%	N	%
Heart disease patients (150)	65	43.33	85	56.66	1	0.66	149	99.33
Control (100)	17	17	83	83	2	2	98	98
Total	82	32.8	168	67.2	3	1.2	247	98.2
	$X^2=18.78$, Df=1, $P \leq 0.05$				$X^2=0.900$, Df=1, $P > 0.05$			

4.3 Distribution of study groups according to sex

The seroprevalence of anti-*Toxoplasma* IgG Abs in present study groups showed revealed in both sexes, and the higher value of anti-*Toxoplasma* IgG Abs was 36 of 81(44.44%) in male of heart disease patients and the higher value of anti-*Toxoplasma* IgG Abs in apparently healthy was showed in male 13 of 55 (23.6%), and the lower value of seroprevalence of anti-*Toxoplasma* Abs 29 Of 69 (42.02%) was in female in heart disease patients, also the lower value of anti-*Toxoplasma* IgG Abs in apparently healthy was showed in female 4 of 45 (8.9%). However, the results of current study indicate to the presence of significant differences at ($p \leq 0.05$) between male and female in the seropositivity of anti-*Toxoplasma* Abs in apparently healthy (negative controls), whereas no significant difference between both gender in heart disease patients in the IgG results at ($p \leq 0.05$), also no significant difference between both gender in the IgM results at ($p \leq 0.05$), table (4-3).

Table 4-3 : Seroprevalence of anti-*Toxoplasma* Abs in heart disease patients and apparently healthy controls distributed according to sex

Number of samples	gender group	ELISA test								
		IgG Positive		IgG Negative		IgM Positive		IgM Negative		Total
		N	%	N	%	N	%	N	%	
Heart disease patients	Male	36	44.44	45	55.55	0	0	81	100	81
	Female	29	42.02	40	49.38	1	1.44	68	98.55	69
		X ² = 0.089, Df=1, P>0.05				X ² = 1.182, Df=1, P>0.05				
Control (100)	Male	13	23.6	42	76.4	0	0	55	100	55
	Female	4	8.9	41	91.1	2	4.6	43	95.5	45
		X ² = 3.815, Df=1, P≤0.05				X ² = 3.061, Df=1, P>0.05				250

4.4 Distribution of study groups (heart disease patients) according to type of clinical investigations (type of heart disease)

Heart disease patients were divided into three groups according to types of heart disease, and the results of the present study showed presence of anti-*Toxoplasma* Abs (IgG) in all types of heart disease patients, and the higher value of anti-*Toxoplasma* IgG Abs was 29 of 52 (55.8%) showed in Mitral stenosis (MS) groups within heart disease patients. Whereas the lower value of anti-*Toxoplasma* IgG Abs in heart disease patients was showed in Myocardiopathy10 of 33(30.3%). However, the results of current study indicated to there is significant difference at ($p\leq 0.05$) among heart disease patients in IgG results, whereas there were no significant difference among heart disease patients in IgM results, table (4-4) explain the details.

Table 4-4: Seroprevalence of anti-*Toxoplasma* Ab in heart disease patients with compared between MS, Myocardopathy and Ischemic heart disease.

Type of Heart disease	ELISA test								Total
	IgG Positive		IgG Negative		IgM Positive		IgM Negative		
	N	%	N	%	N	%	N	%	
Myocardopathy	10	30.3	23	69.7	0	0	33	100	33
MS	29	55.8	23	44.2	0	0	52	100	52
Ischemic Heart Disease	25	38.5	40	61.5	1	1.5	64	98.5	65
	X ² =6.181, Df=2, P≤ 0.05				X ² =1.151, Df=2, P>0.05				150

4.5 Distribution of study groups according to age groups

According to age the samples of heart disease patients with age rating 18 and 82 years had been divided into five groups and the apparently healthy controls with age rating 16 and 56 years had been divided into three groups. The results of this study showed that presence of anti-*Toxoplasma* Abs (IgG) in all age groups of both groups (heart disease patients and controls), the highest value of anti-*Toxoplasma* IgG Abs was 24 of 38 (63.2%) showed in age group (61-75) within heart disease patients. Whereas the lowest value of anti-*Toxoplasma* IgG Abs in heart disease patients was showed within age group (15-30) 6 of 19 (26.3%). Also the highest value of anti-*Toxoplasma* IgG Abs in apparently healthy controls was 8 of 41 (19.5%) showed in age group (31-45). Whereas the lower value of anti-*Toxoplasma* IgG Abs in apparently healthy controls was showed within age group (15-30) 2 of 23 (8.6%). The results indicate to there was significant differences at $p \leq 0.05$ among the age groups in the seropositivity of anti-*Toxoplasma* Abs (IgG) results in heart disease patients, while no significant differences among the age groups in the seropositivity of anti-*Toxoplasma* Abs (IgG) results in apparently healthy

controls, also there is no significant difference at ($p \leq 0.05$) among heart disease patients in IgM results, table (4-5)

Table 4-5 : Seroprevalence of anti-*Toxoplasma* Abs (IgG & IgM) in heart disease patients and apparently healthy controls distributed according to Age

Number of samples	Age group	ELISA test								Total
		IgG +		IgG-		IgM +		IgM-		
		N	%	N	%	N	%	N	%	
Heart disease patients (150)	15-30	5	26.3%	14	73.7%	0	0%	19	100%	19
	31-45	8	32%	17	68%	0	0%	25	100%	25
	46-60	24	39.3%	37	60.7%	1	1.6%	61	98.3%	61
	61-75	24	63.2%	14	36.8%	0	0%	37	100%	38
	76-82	3	42.9%	4	57.1%	0	0%	7	100%	7
		X ² =9.915, Df=4, P≤0.05				X ² =1.429, Df=4,P≥0.05				150
Control (100)	15-30	2	8.6%	21	91.3%	0	0%	23	100%	23
	31-45	8	19.5%	33	80.4%	1	2.4%	40	97.5%	41
	46-56	7	19.4%	29	80.5%	1	2.7%	35	97.2%	36
	Total	X ² =1.460, Df=2, P≥0.05				X ² =0.609, Df=2, P≥0.05				100

4.6.Distribution of study groups infected with toxoplasmosis according to blood groups.

Heart disease patients and apparently healthy controls were divided into four groups according to types of blood group, the results showed presence of anti-*Toxoplasma* Abs (IgG) in all types of blood groups, with highest prevalence among Heart disease patients and controls with blood group AB⁺ 29 (67.4%) and 9 (34.6%) respectively, whereas the lowest value in Heart disease patients and control of group A⁺ 11 (31.4%) and 2(9.5 %) respectively. The results indicated to there was significant differences among

the blood groups in the seropositivity of anti-*Toxoplasma* Abs (IgG) results in both subjects, as in table (4-6).

Table 4-6 : Seroprevalence of anti-*Toxoplasma* Ab (IgG) in heart disease patients and controls distributed according to Blood groups

Test subject	Blood groups	Anti-Toxoplasma Abs+(IgG)		Anti-Toxoplasma Abs-(IgG)		Total
		N	%	N	%	
Heart disease patients (150)	O⁺	12	32.4	25	67.6	37
	A⁺	11	31.4	24	68.4	35
	B⁺	13	37.1	22	62.9	35
	AB⁺	29	67.4	14	32.6	43
Statistical		X² =14.53 , df=3, P ≤ 0.05				150
Control (100)	O⁺	3	10.3	26	89.7	29
	A⁺	2	9.5	19	90.5	21
	B⁺	3	12.5	21	87.5	24
	AB⁺	9	34.6	17	65.4	26
Statistical		X² =7.804, df=3, P≤0.05				100

4.7 Distribution of study groups infected with toxoplasmosis according to the habitation.

In relation to inhabitation which living both groups (heart disease patients and controls), the results of current study showed significantly high positive percentage at $p \leq 0.05$ of anti-*Toxoplasma* Abs(IgG) in heart disease patients residence in rural area where 42(52.5%) in compare with urban residents 24(34.3%). Also the results of current study showed significantly high positive percentage at $p \leq 0.05$ of anti-*Toxoplasma* Abs(IgG) in controls inhabited rural area where 16(32%) in compare to urban residents 3(6%), as summarized in table (4-7).

Table 4 -7 : Seroprevalence of anti-*Toxoplasma* Abs (IgG & IgM) in heart disease patients and controls were distributed according to habitation

Test subject		ELISA test				Total
		Anti-Toxoplasma positive		Anti-Toxoplasma negative		
		N	%	N	%	
Heart disease patients	Urban	24	34.3	46	65.7	70
	Rural	42	52.5	38	47.5	80
Statistical		X ² =4.375, df=1, p≤0.05				
Control	Urban	3	6	47	94	50
	Rural	16	32	34	68	50
Statistical		X ² =8.575 ,df=1 ,p≤0.05				

4.8 Incidence of Infected study groups with Toxoplasmosis According to Contact with Cats

The results of present study confirmed that seroprevalence of toxoplasmosis was significantly associated with close contact with cats of heart disease patients infected with toxoplasmosis ($p\leq 0.05$) they recorded 51 (54.8%) were in contact with cats and 15 (26.3%) were not in contact with cats within heart disease patients having IgG *Toxoplasma* Abs , whereas in apparently healthy controls (having IgG *Toxoplasma* antibodies) they recorded 17(23.7%), were in contact with cats and 2(7.1%) were not in contact with cats. The results indicated to there was significant differences among the subjects in the seropositivity of anti-*Toxoplasma* Abs (IgG) results in heart disease patients and controls, as in table (4-8).

Table 4-8: Seroprevalence of anti-*Toxoplasma* Abs (IgG & IgM) in heart disease patients and controls distributed according to Cat exposure

Test subject	Cat exposure	ELISA Test				Total
		Anti-Toxoplasma positive		Anti-Toxoplasma negative		
		N	%	N	%	
Heart disease patients	Yes	51	54.8	43	45.2	93
	No	15	26.3	42	73.7	57
Statistical		X ² =10.842, df=1, P≤0.05				150
Control (apparently healthy)	Yes	17	23.7	55	76.3	72
	No	2	7.1	26	92.9	28
Statistical		X ² =2.768 , df=1,P≤0.05				100

4.9 Evaluation of anti and pro-inflammatory interleukins level in heart disease patients and controls.

In the present study the level of pro-inflammatory and anti-inflammatory interleukins (IL-12, IL-10 and IL-17) were estimated in both group (heart disease patients and controls) by ELISA, and showed increased in the level of IL-12 (82.85pg/ml) in heart disease patients comparing with controls was appeared (60.97pg/ml) and there was significant difference $p < 0.05$ between them, interleukin 17 concentration was (16.68pg/ml) in heart disease patients in comparing to controls was (20.01pg/ml), and there was no significant difference $p < 0.05$ between them. On the other hand the results of IL-10 was (7.18 pg/ml) in heart disease patients in comparing with apparently healthy controls was (8.14pg/ml) showed no significant difference between heart disease patients and controls, as show in table (4-9).

Table 4-9 : The interleukins level in heart disease patients with seropositive toxoplasmosis and controls

Interleukins	Groups	Mean	Std. Deviation
IL12	Patients(Anti- <i>Toxoplasma</i> positive)	82.85*	58.95
	Controls(Anti- <i>Toxoplasma</i> negative)	60.97	17.23
	t= 2.382, significant P value =0.020		
*There is a significant difference between groups at α 0.05			
IL17	Patients(Anti- <i>Toxoplasma</i> positive)	16.68	13.62
	Controls(Anti- <i>Toxoplasma</i> negative)	36.01	17.48
	t= -2.127, significant P value =0.146		
There is no significant difference between groups at α 0.05			
IL10	Patients(Anti- <i>Toxoplasma</i> positive)	7.18	3.71
	Controls(Anti- <i>Toxoplasma</i> negative)	8.14	2.30
	t= -1.299, significant P value =0.199		
There is no significant difference between groups at α 0.05			

4.10 Evaluation of anti and pro-inflammatory interleukin levels in positive and negative anti-*Toxoplasma* .

In this study the interleukins measured in controls whose have anti-*Toxoplasma* Abs, and who was not have anti-*Toxoplasma* ,and the results indicate to the presence of significant difference at ($p < 0.05$) in the level of IL-12 between Anti-*Toxoplasma* positive (IL-12 = 220.24pg/ml), and Anti-*Toxoplasma* negative (IL12 = 60.97 pg/ml). While the results of IL-10 in Anti-*Toxoplasma* positive was (IL-10 = 9.14pg/ml) and IL-10 in Anti-*Toxoplasma* negative was (8.14pg/ml) ,and the results of IL-17 in Anti-*Toxoplasma* positive showed (IL-17=64.03pg/ml), whereas in Anti-*Toxoplasma* negative was (IL-17=36.72 pg/ml). So that there was no

significant difference in IL-10 and IL-17 between both groups, as in table (4-10).

Table 4-10 :The interleukin levels among control subjects whom have anti-*Toxoplasma* Abs and who was not have anti-*Toxoplasma* Ab.

Groups		Mean	Std. Deviation
IL12	Anti- <i>Toxoplasma</i> positive	220.24**	165.96
	Anti- <i>Toxoplasma</i> negative	60.97	17.23
	t= 4.052, significant P value =0.001		
*There is a significant difference between groups at α 0.05,0.01			
IL17	Anti- <i>Toxoplasma</i> positive	64.03	46.84
	Anti- <i>Toxoplasma</i> negative	36.72	41.48
	t= 1.893, significant P value =0.067		
There is no significant difference between groups at α 0.05			
IL10	Anti- <i>Toxoplasma</i> positive	9.14	2.74
	Anti- <i>Toxoplasma</i> negative	8.14	2.30
	t= 1.207, significant P value =0.236		
There is no significant difference between groups at α 0.05			

4.11 Correlation coefficient

The results of correlation coefficient indicate to little correlation between anti-*Toxoplasma* IgG and IL-10 and same one between anti-*Toxoplasma* IgM and IL-12 and there was no correlation between Abs and IL-17 . For specify the role of interleukin in heart disease patients infected with toxoplasmosis and the results of correlation coefficient also was indicate to significant correlation at the α 0.05 level between interleukin 17 level and interleukin 10 among heart disease patients(anti-*Toxoplasma* positive), as show in table (4-11).

Table 4-11 : The Correlation coefficient between interleukins level and anti-*Toxoplasma* Abs IgG & IgM in patients with heart disease

Parameter	IL10	IL17	IL12	IgG	IgM
IL10	1				
IL17	0.309	1			
IL12	-0.024	0.274	1		
IgG	0.215	- 0.040	- 0.032	1	
IgM	0.009	- 0.003	0.032	0.196	1
*. Correlation is significant at the α 0.05 level					

The results of correlation coefficient also was indicate to significant correlation at the α 0.05 level between interleukin 17 level and interleukin 12 in controls whom infected with toxoplasmosis(Anti-*Toxoplasma* positive), as in table (4-12).

Table 4-12 : The Correlation coefficient between interleukins level and anti-*Toxoplasma* Abs in controls whom infected with toxoplasmosis(Anti-*Toxoplasma* positive).

parameter	IL-10	IL-17	1L-12	IgG	IgM
IL-10	1				
IL-17	0.261	1			
IL-12	0.268	0.703**	1		
IgG	- 0.446	-0.324	-0.426	1	
IgM	0.337	-0.004	0.393	-0.425	1
*.Correlation is significant at the α 0.05 level					

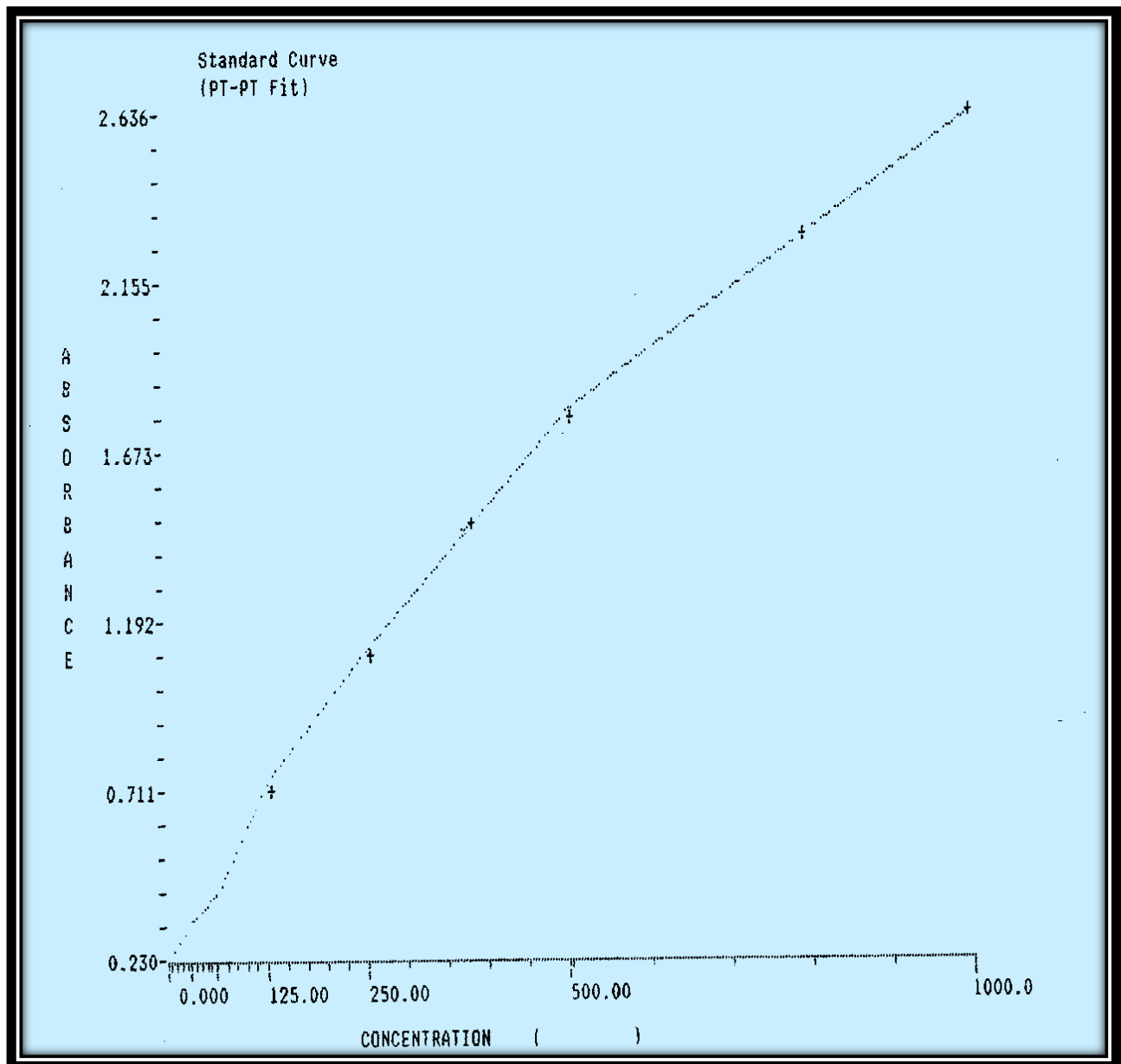


Figure 4-1: Standard curve by which concentrations of IL-12 in the sera of person infected and non-infected were calculated as pg/ ml on X-axis versus absorbency on Y-axis.

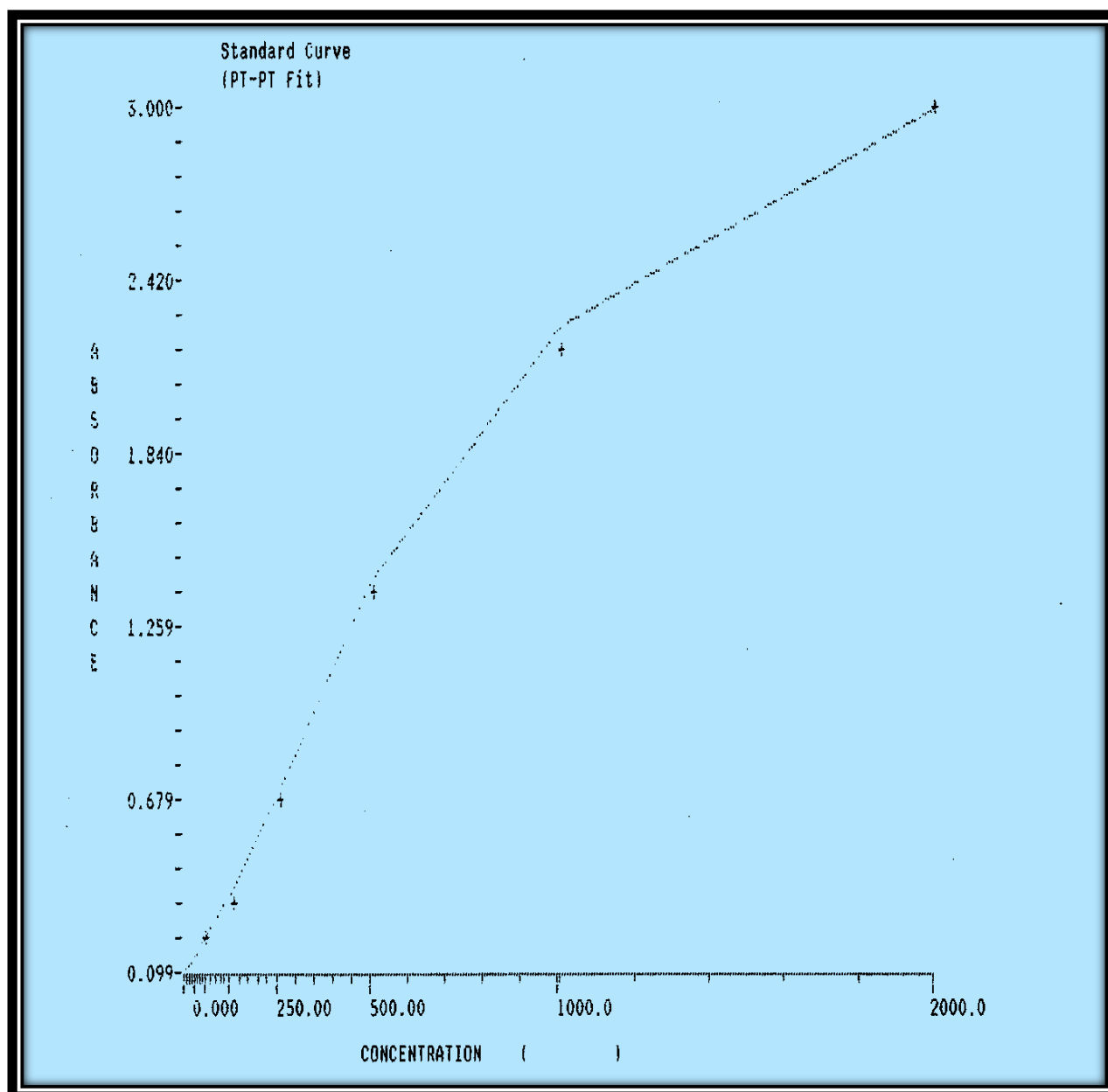


Figure 4-2 : Standard curve by which concentrations of IL-10 in the sera of person infected and non-infected were calculated as pg/ ml on X-axis versus absorbency on Y-axis.

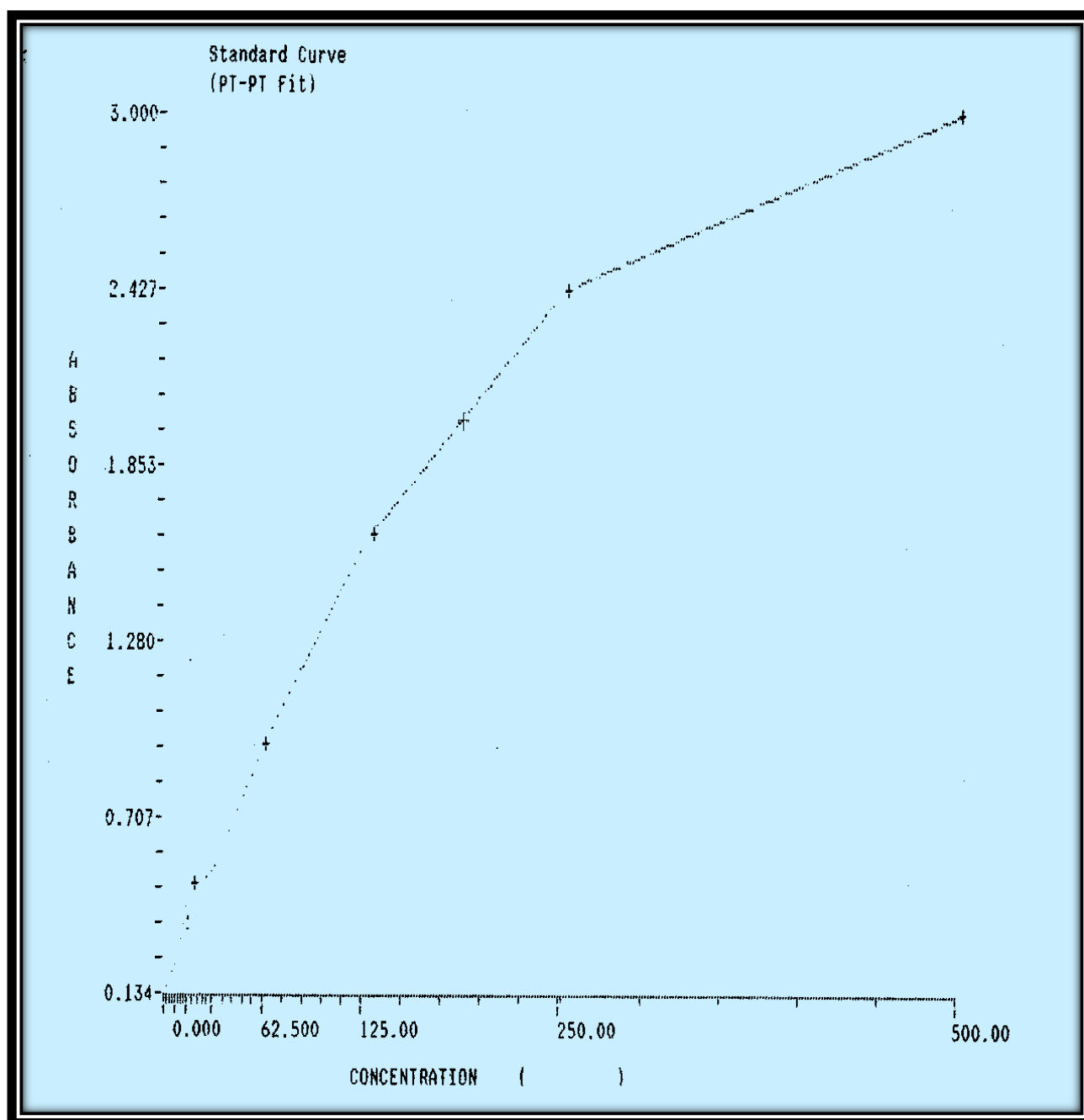


Figure 4-3: standard curve by which concentrations of IL-17 in the sera of person infected and non-infected were calculated as pg / ml on X-axis versus absorbency on Y-axis.

4.12 Estimation of some blood parameters in both groups infected and non-infected with Toxoplasmosis

The present study evaluates the rate of some blood parameters, in infected and non-infected (heart disease patients and controls), there was significant differences at $p \leq 0.05$ between infected and non-infected heart disease patients were observed in mean of eosinophil's and lymphocytes number of infected heart disease patients with toxoplasmosis were more than number of non-infected heart disease patients. While there was no significant difference at $p \leq 0.05$ observed in mean of basophiles, total count of leukocytes (WBCs), neutrophils, monocytes and haemoglobin level (Hb) numbers in both subjects. as shown in table (4-13). Also the results indicates to there was significant difference at $p \leq 0.05$ between infected and non-infected apparently healthy controls were observed in mean of eosinophil's. While there was no significant difference at $p \leq 0.05$ observed in mean of basophiles, total count of leukocytes (WBCs), neutrophils, monocytes, lymphocytes and haemoglobin level (Hb) numbers in both subjects, table (4-14) explain the details.

Table 4-14 : Percentage of some blood parameters in both groups infected and not infected with toxoplasmosis

Blood parameter	Control	No.	Mean	t	P	Heart disease patients	No.	Mean	t	P
Hb	Infected	17	13.5	-1.2	0.21	Infected	65	13.42	-1.07	0.28
	Non infected	41	14.1			Non infected	85	13.78		
WBC	Infected	17	11.23	1.90	0.65	Infected	65	9.225	-1.03	0.303
	Non infected	41	9.288			Non infected	85	9.904		
Monocytes	Infected	17	0.730	1.5	0.12	Infected	65	0.601	-1.2	0.23
	Non infected	41	0.552			Non infected	85	0.678		
Basophils	Infected	17	0.094	1.0	0.28	Infected	65	0.092	-0.28	0.77
	Non infected	41	0.080			Non infected	85	0.095		
Eosinophil's	Infected	17	1.22	2.5	0.02	Infected	65	1.201	3.77	0.001
	Non infected	41	0.347			Non infected	85	0.325		
Neutrophils	Infected	17	6.738	1.8	0.68	Infected	65	5.393	-0.5	0.61
	Non infected	41	5.282			Non infected	85	5.66		
Lymphocyte	Infected	17	2..97	-0.2	0.76	Infected	65	2.681	-2.19	0.03
	Non infected	41	3.08			Non infected	85	3.099		

Chapter

Five

Discussion

5. Discussion

Toxoplasmosis is an endemic infectious disease, very distributed in all fragments of the world, but clinically uncommonly causes significant disease. The current study was performed in Thi-Qar province to evaluate the role of cytokines in Heart disease patients infected with toxoplasmosis and estimate the prevalence of toxoplasmosis among heart disease patients.

5.1 Seroprevalence of anti-*Toxoplasma* Abs in study groups

The seropositivity of anti-*Toxoplasma* Abs that scored by ELISA in the current study was (44%) in heart disease patients are higher than those in apparently healthy controls (19%), the percentage of anti-toxoplasmosis Abs in overall subjects was (34%). According to the results that obtained by Zhang *et al.*, (2015) which scored all patients in the intensive care unit (ICU) including patients suffering from heart disease are usually considered to be particularly immunocompromised; they may be very susceptible to the reactivation of *T.gondii* infection. Also the results by Gur,*et al.*, (2006) demonstrated this patient group was immunocompromised because of the underlying diseases so they were at risk of opportunistic infections.

Thus, detection and investigation of anti- *Toxoplasma* antibodies are of great interest, especially in ICU patients presenting with at least one form of organ failure (Demar, *et al.*,2007). The studies mentioned above explained the reason for the difference in the percentage of toxoplasmosis in heart disease patients was more than those in controls. The results in the present study was higher than those obtained by several researchers from different regions in Iraq by Al-Ghezy (2012) and Al-Mosawi (2014) done in the same province which recorded (23% and 21.94%) respectively by using ELISA test, Hasson, (2004); Dargham, (2011) in Al- Najaf and Saleh, (2011) which recorded 19.7%, 26.8% and 20.54% respectively in others provinces. Other study done in Baghdad by Al-Dalawi, (2007) and Al-

Kaise and Ali (2010) which recorded (29.2% and 22%) respectively. Also the results of current study was disagreed with several previous studies in some Arab countries in Saudi Arabia by Al-Qurashi *et al.* (2001) and in Sudan by Elamin *et al.*, (2012) which recorded 20.7% and 21.8% respectively.

Moreover current study was disagreed with several previous studies in world, in Mexico by Galvain-Ramirez *et al.*, (2010) and in Iran by Hashemi and Saraei (2010), the seroprevalence was 22.4% and 22.6% respectively by using ELISA. Whereas the results of our study was matched with other finding by Salih, (2010); AL-Mousawi and Shani, (2011); Al-Ubaydi, (2011) and Al-Abudy, (2014) which recorded high percentage of seropositive anti-*Toxoplasma* Abs 39.6%, 45%, 32.81% and 33.5% respectively from different regions in Iraq. While the results of current study lower than seroprevalence of anti-toxoplasmosis that reported in Somalia Zard *et al.* , (1980), in Kuwait AL-Nakib *et al.*, (1983) and in Iran by Sharif *et al.*, (2010) which recorded seropositive toxoplasmosis Ab (53%, 58.2% and 77%) respectively.

The seroprevalence estimated for human population varies greatly among different countries, among different geographical regions in the same country, even within, same city (Jones and Dubey, 2010). this may explain the variation in seropositivity. Also the similarities and variation in the results may be related to several factors, including climatic, nutrition habits, cultural patterns, sample size, target population, age , sampling method, types of laboratory tests and tools (Etheredge and Frankel , 1995; Dubey, 2010a) or may due to different manufacture origin of the kits used (Al-Saadii, 2013).

5.2 Distribution of study groups according to anti-*Toxoplasma* Abs in serum

In the study indicated that anti-*Toxoplasma* Abs (IgG) was (43.33%) in heart disease patients and (17%) of apparently healthy controls, the percentage of anti-toxoplasmosis Abs (IgG) in overall subjects was (32.8%), are higher than those during acute infection (IgM) was (1.2%), this was higher than results of anti-toxoplasmosis Ab(IgG) of other studies in the same city by Al-Ghezy, (2012); Al-Abudy, (2014) and Al-Mosawai, (2014) who indicated that the seroprevalence IgG *Toxoplasma* Abs was 17.5%, 27.5% and 11.91% respectively, while the seroprevalence IgM *Toxoplasma* in our study was lower than the seroprevalence IgM in same city by Al-Ghezy, (2012); Al-Abudy, (2014) and Al-Mosawai, (2014) who indicated that the seroprevalence IgM *Toxoplasma* antibodies was 3.4%, 3.44% and 5.33% respectively.

The result of current study was matched with other finding by Tallab *et al.*,(2012) who recorded that the rate of prevalence anti-*Toxoplasma* Abs in heart disease patients was 37.9% and Al-Aboody, (2015) who scored that the rate of prevalence of anti-*Toxoplasma* Abs in males blood donors was 35.3%, while the results of ELISA-IgM test by Al-Aboody, was 2.7%. The was was corresponding with previous studies in other provinces in Iraq by Tawfeeq *et al.*,(2012); Mahmood, *et al.*,(2013) and Alkhanak *et al.*,(2015) which recorded that the seroprevalence IgG *Toxoplasma* antibodies was 32.4%, 30.25% and 35.31% respectively, while result of ELISA-IgM test by Mahmood *et al.*, (2013) was 2.5%. Globaly the seroprevalence of toxoplasmosis varies in different regions such as in Mexico, Alvarado-esquivel *et al.*, (2016) who indicated the seroprevalence IgG *Toxoplasma* Abs was 13.8% within heart disease patients. The results in the present study could be explained by the detail that the group examined consisted of

heart disease patients and apparently healthy persons, and IgG positive persons were infected with latent toxoplasmosis without a persistence of IgM antibodies after acute infection in the past (Carmen *et al.*, 2006).

5.3 Distribution of study groups according to gender

The seroprevalence of anti-*Toxoplasma* Abs (IgG and IgM) in the present study among males was 36 of 81 (44.44%) are higher than those in females 28 of 69 (42.02%) within heart disease patients, whereas the seroprevalence of anti-*Toxoplasma* Abs (IgG and IgM) in apparently healthy controls was 13 of 55 (23.6%) among males are higher than those in females 4 of 45 (8.9%). This finding is corresponding with other previous studies by Frenkel and Ruiz, (1980); Coelho *et al.* (2003) and Yassin, (2015) which found higher percentage of seropositivity in males (66%, 79% and 71.4%) than females (58%, 63.4% and 28.6%) respectively. In contrast other studies by Hamadato *et al.* (1997); Xiao *et al.* (2010) and Kheirabadi *et al.*, (2013) which found the seroprevalence of anti-*Toxoplasma* Abs in females higher than males. Also the results of current study was disagreed with other study in same province by Al-Mosawai, (2014) who recorded the seropositivity of anti-toxoplasmosis Abs in males was (7.52%) whereas (14.42%) in females. Whereas other previous studies showed no significant difference between males and females in seroprevalence of toxoplasmosis (Galvain-Ramirez *et al.*, 2010; Aboelhadid *et al.*, 2013). This may be due to many factors including lower immunity to *T.gondii* in males as compared to females, social activities and different occupational. Also Females are more resistant than males to parasitic infection because of testosterone immunosuppressive properties and gender associated differences in exposure (Morales-Mortar *et al.*, 2004). Levels of immunoglobulin's including IgM, IgG in females are higher than those in males with toxoplasmosis (Al-Qureshi, 2004).

5.4 Distribution of study groups according to the types of heart disease patients

According to clinical investigations , cardiac stress testing, coronary computed tomographic angiography, coronary angiogram and electrocardiograph, heart disease patients were suffering from Mitral stenosis (MS), Myocardopathy and Ischemic heart disease (IHD). The results of present study was demonstrated the association between seroprevalence of anti-toxoplasmosis Abs and heart disease patients was significantly. We found a 44% seropositive of anti-*Toxoplasma* Abs in patients suffering from heart disease. This results was higher than other study in same province by Tallab, *et al.* (2012) who recorded that seroprevalence of anti-toxoplasmosis in heart disease patients was (37.9%). Also our study was higher than other universal study in Mexico by Alvarado-Esquivel *et al.*,(2016) and in China by (Zhang *et al.*, 2015) who recorded that seroprevalence of anti-toxoplasmosis in heart disease patients was (13.8%) and (11.58%) respectively.

Whereas this results was lower than other study in Turkey by (Gur,*et al.*, 2016) who recorded that seroprevalence of anti-toxoplasmosis in heart disease patients was(68%). The results of present study showed presence of anti-toxoplasmosis Abs in all types of heart disease patients with significant difference among them, and the highest percentage of anti-toxoplasmosis Abs in(MS) was 55.8% , and the lower value was 30.3% at Myocardopathy. Whereas the percentage of anti-toxoplasmosis Abs in IHD was 38.5%. This results was higher than other results by Alvarado-Esquivel *et al.*,(2016) who recorded the seroprevalence of anti-toxoplasmosis in IHD and Myocardopathy was (16.7% and 10.5%) respectively. Whereas the results by Tallab *et al.*,(2012) which scored the rate of percentage of anti-toxoplasmosis in IHD was (37.9%). In transplant

patients, severe of disseminated toxoplasmosis can result from either reactivation of latent infection in the recipient or infection from a cyst-containing organ from a seropositive donor given to seronegative Recipient (Martina *et al.*, 2011). However the results of current study indicate to that patients with heart disease represent a risk group for *T. gondii* infection. The difference in the percentage of anti-toxoplasmosis Abs among types of heart disease patients may be due to a small sample size of some subgroups of specific diagnosis.

5.5 Distribution of study groups according to age

The results of present study revealed there are significant differences between the age groups and the percentage of anti-*Toxoplasma* Abs in heart disease patients with highest value was (63.2%) at age group of (61-75) years, and the lowest one was (26.3%) at age group of (15-30) years. This result was disagreement with the data obtained in same province by AL-Mosawi, (2014) and AL-Aboody, (2015) which scored that the highest value of seropositivity of toxoplasmosis was between (20-30) years. Also our results were different from other finding by AL-Dalawi, (2007); Mahmood *et al.* , (2013) and Al-Hindi *et al.* , (2016) who found that the highest value of seropositivity of anti-toxoplasmosis was between (15-30) years. Whereas this results was coincided with other studies by Alvarado-Esquivel *et al.*, (2016) who was found that the highest value of seropositivity of anti-toxoplasmosis was between (50-70) years among heart disease patients, and Alvarado-esquivel *et al.*, (2006) who was found that the highest value of seropositivity of toxoplasmosis was between (55-64) years within psychiatric inpatients. Also the result was coincided with several previous study that indicated the percentage of anti-*Toxoplasma* Abs was increased with age (Jones *et al.*, 2001 and Kolbekova *et al.*, 2007).

On the other hand the present study revealed there is no significant differences at $p \leq 0.05$ between the age groups and the percentage of anti-*Toxoplasma* Abs in apparently healthy controls with highest value was (19.5%) at age group of (31-45) years, and the lowest one was (8.6%) at age group of (15-30) years. This result was discrepantly with the data obtained by AL-Dalawi, (2007); AL-Rawi, (2009) AL-Khozai and Khadem, (2011); Mahmood *et al.*, (2013); by; AL-Mosawi, (2014) and AL-Hindi *et al.*, (2016) which found that the highest value of seropositivity of anti-toxoplasmosis was between (20-30) years. Whereas this results was agreement with other results by Al-Hamdani and Al-Mahdi, (1996) ; Kadhim Ibadi and Hamedon, (2015) and Zhang *et al.*, (2015) which found that the highest value of seropositivity of anti-toxoplasmosis was between (30-45) years.

These findings showed that all age groups may infected with *T. gondii* according to the probability of the exposure to the oocysts of the parasite, its virulent and the immunity of the individual. These differences between all previous and current studies may be due to the differences in the sensitivity and specificity of test used for diagnosis of differences in the age of the studies groups and the response of each host to the strain of parasite ,the variation in parasite strains may play an important role in the stimulation of host immune response against the parasite (Suzuki and John ,1994). The percentage of toxoplasmosis increases with age in heart patients.

5.6 Distribution of study groups according to blood groups

The current study confirmed the relation between blood group system and toxoplasmosis with highest seroprevalence among heart disease patients and apparently healthy controls of blood group AB⁺ (67.4%) and

(34.5%) respectively, whereas the lowest prevalence in samples of blood group A⁺ (31.4%) and (9.5%) within both subjects. This result disagrees with previous study by Al-Dujaily (1998) and Al-Khaffaf (2001), who recorded that the highest prevalence among samples of blood group A⁺. Also this result different from other finding by Al-Shikhly *et al.* (2010) who found that the higher ratio of seroprevalence toxoplasmosis among female (30.6%) of B⁺ blood group; Al-Shikhly *et al.* (2013) and Al-Abudy (2014) who revealed that the higher percentage result (28.8% and 42.5% respectively) respectively with blood group O⁺. Other studies appeared no significant relation between *T. gondii* and ABO factors (Rodrigues *et al.*., 2011 and Mohammed *et al.*, 2013). Whereas our finding was matched with other previous studies by Zhiburt *et al.*, (1997); Al-Saadii, (2013) and Mahmood, *et al.*, (2013) who revealed that seropositivity of anti-*Toxoplasma* Abs were concentrated on blood group type AB⁺.

Four previous studies by Midfvedt and Vaage, (1989); Lopez *et al.*, (1993); Zhiburt *et al.*, (1997); Kolbekova *et al.*, (2007) refer to a relationship between the B and AB blood group and the presence of anti-*T. gondii* antibodies, in contrast two other similar investigations did not find any evidence of this association (Gill 1985; Lecolier *et al.*, 1990). The suggestion that the B antigen represents a receptor for *T. gondii* does not appear to be valid in different population (Rodrigues *et al.*, 2011). The present result of this study is a possibility that the parasite utilized glycoconjugates, which characterize the blood phenotypes of the ABO blood group system, as a potential receptors. These glycosylated molecules are expressed in the gastrointestinal tract (GIT), which is also utilized as main route of *T. gondii* infection (Carruthers *et al.*, 2000). Additionally, there is biochemical evidence that several microorganisms utilize biochemical evidence that several microorganisms utilize glycoconjugates as receptor (Hooper and Gordon, 2001).

The result of negative blood group phenotypes (A-, B-, AB- and O-) were not considering in this study, because of their small size number.

5.7 Distribution of study groups according to the habitation

The results in our study showed the percentage of seroprevalence anti-*Toxoplasma* Abs in heart disease patients inhabited rural area was (52.5%) are higher than those inhabited urban area was (34.3%). Also the percentage of seroprevalence anti-*Toxoplasma* Abs in apparently healthy controls inhabited rural area was (32%) are higher than those inhabited urban area was (6%). This finding was corresponding with other previous study in same province by Al-Aboody (2015) who was found significantly higher ($p < 0.05$) seropositive rural than urban. Also the results of present study was matched with previous studies in other countries by Zoe *et al.*, (2009) in Romania and Sroka *et al.*, (2010) in Poland whom found that human living in rural area had significantly greater ratio of toxoplasmosis with (63.68% and 59%) compared to urban residents (55.12% and 41%) respectively. The present result was disagreed with other previous studies by Al-Jubori, (2005); Al-Najjar, (2005); Al-Wattari, (2005); Karem, (2007) and Jassam, (2010) revealed no significant difference between seroprevalence of anti-*Toxoplasma* Abs and habitation. Also current results disagreed with previous study by Alvarado-Esquivel *et al.*, (2016) who found that was difference in seroprevalence of anti-*Toxoplasma* Abs between urban (14.6%) than in rural regions (7.4%). Therefore, the habitation are considered a high-risk factor for toxoplasmosis. In general, the differences in the infection rates of toxoplasmosis between urban and rural residents within same country may be related to many factors like socio-economic status which related to oocyst shedding from cats, climate, nutritional behavior (increasing trend of raw vegetable consumption), health-related practices and transmission route (Cantos *et al.*, 2000; Furtado *et al.*, 2011).

5.8 Distribution of study groups according to Contact with cats

The results of current study showed seroprevalence of *T.gondii* antibodies was (54.8%) significantly higher among heart disease patients who cats exposed in compared with those who did not (26.3%). Also the seroprevalence of *T.gondii* antibodies was (23.7%) significantly higher among apparently healthy controls who cats exposed in compared with those who did not (7.1%) The association between exposure to cats and seroprevalence of *T. gondii* antibodies that found in the present study was strong.

We demonstrated there was in agreement with previous study in same province by Al-Mosawai, (2014) who found that seroprevalence of *T.gondii* antibodies was significantly higher among subjects who cats exposed in compared with those who did not. Also this results was matched with other studies by (Othman, 2004; Alvarado-esquivel *et al.*, 2006; Esquivel *et al.*, 2007; Karem, 2007; Salih, 2010; Tawfeeq *et al.*, 2012 and Mohammed *et al.*, 2013).While disagreement was found with the results reported by (Fallah *et al.*,2008; Al-Aubady, 2011 and Abdel-Raouff and Elbasheir, 2014). The results in the present study could be estimated a strong association between seropositivity and exposure to cats, that may be cats are widely distributed in Thi-Qar province, this may increase chance of contact with cats litter or contaminated food or water by parasite Oocytes. Felids are the only definitive hosts responsible for shedding Oocytes that contaminate the environment and become infective for long time in water or soil (Dubey, 2010 a).

5.9. Evaluation of anti-inflammatory and pro-inflammatory interleukins (IL-12,IL-10 and IL-17) level in both groups

In the present study, the results showed elevating in the level of IL-12 in heart disease patients and apparently healthy controls, whom infected with toxoplasmosis comparing with non-infected with toxoplasmosis, this results was matched with results of other studies Abdul-lateef *et al.*,(2012); Alkhanak *et al.* ,(2015) which found increased in the level of IL-12 in persons with asymptomatic toxoplasmosis in comparison with free-toxoplasmosis persons. Also the results in present study was matched with other previous study by Gomes *et al.*, (2014) who scored increased in the level of IL-12 in persons infected with *Toxoplasma* in skeletal muscle cells (SkMC), and the present study was different from others Al-Khafajhi *et al.*, (2011) who found reduced in the level of IL-12 in women infected with toxoplasmosis in comparison with free-toxoplasmosis women.

Production of IL-12 and IFN- γ is essential to control infection by *T. gondii*, IFN- γ synergizes with IL-12 to drive the differentiation of Thp to Th1 phenotype, express IL-12 receptor on T cells, and inhibit the antagonist IL-4 to prevent the differentiation of Thp towards Th2 phenotype (Cordeiro *et al.* ,2008). The role of IL-12 in toxoplasmosis including induces production of IFN- γ from natural killer (NK) cells and T cells, IFN- γ signaling promotes the development of a number of activator of transcription 1 (STAT1)-dependent anti-parasitic effector mechanisms, including reactive oxygen intermediates (ROI) production and p47 GTPase upregulation (Tait and Hunter, 2009). Results of current study showed no significant difference between heart disease patients and controls in the mean serum level of IL-17, also showed a decrease in the mean serum level of IL-17 in heart disease patients with toxoplasmosis seropositive when compared with its serum levels in controls, and this may be because the

increasing level of IL-12 that cause dysregulated for IL-17, this results was matched with other previous study by (Sauer *et al.*, 2013) who found that the level of IL-17 was decreased in patients with Ocular toxoplasmosis, and also matched with other results by (Mohammed, *et al.*, 2012) who found decreased the level of IL-17 in aborted women after treated comparing with acute toxoplasmosis.

Whereas the results of current study showed the levels of IL-17 in apparently healthy controls infected with toxoplasmosis was higher than those in apparently healthy controls non-infected with toxoplasmosis, this results was matched with other study by Al-Dahmoshi *et al.*, (2013) who reported the level of IL-17 was increased in aborted women. Also the increase in level of IL-17 in the current result match with other researchers (Gazzinelli, *et al.*, 1993) who found that increase in IL-17 had been scored in early stage of infection. The role of IL-17 is controversial, some studies indicate the role of IL-17 in toxoplasmosis involved in the development and early recruitment of neutrophils, which are essential to clear the parasites during initial stages of infection (Yisong and Richard, 2009). However, in the current study the role of IL-17 may be dysregulated and provoke toxoplasmosis in heart disease patients towards chronicity.

On the other hand, the results of IL-10 showed no significant difference between heart disease patients and controls and this may be because the increasing level of IL-12 that have antagonist effect with IL-10. The results of correlation coefficient indicate to little correlation between anti-*Toxoplasma* IgG and IL-10, this low level of IL-10 during chronic or past infection with toxoplasmosis in the current study matched the results of other studies (Al-Khafajhi *et al.*, 2011 and Abdullah *et al.*, 2011) who found reduced in the level of IL-10 in women infected with toxoplasmosis in comparison with free-toxoplasmosis women, IL-10 plays a vital role in

controlling the inflammatory response during chronic phase of *T. gondii* infection (Wilson and Wille-Reece, 2005). IL-10 serves a dual important role during acute toxoplasmosis, by it inhibits IFN- γ production and the proliferation of T-lymphocytes, thus preventing a potentially protective Th1 immune response. Such T-cell-dependent immune suppression exerted by IL-10 primarily appears to avoid overwhelming inflammation which eventually leads to death. IL-10 may also deactivate macrophages, thus reducing IFN- γ -induced toxoplasmodicidal activity and facilitating intracellular parasite survival. Hence, IL-10-induced immune suppression following infection with *T. gondii* is beneficial for both the parasite and the host and favors a stable host - parasite relationship (Ellis-Neyer *et al.*, 1997).

Concerning relation of heart disease and toxoplasmosis, the present finding was compatible with finding of Hidron *et al.* (2010), who found that *T. gondii* is the reason for myocarditis and pericarditis, *T. gondii* is the second relevant pathogen in protozoan myocarditis (Feldman and McNamara, 2000; Kirchoff *et al.*, 2004), whereas this finding is incompatible with finding of Tallab, *et al.* (2012), who found there is no any relationship between *T. gondii* and Atherosclerosis. Also the results in present study coincided with other results by Gur *et al.*, (2006); Zhang *et al.*, (2015) and Alvarado-Esquivel *et al.*, (2016) which found the association between toxoplasmosis and heart disease was highly significant. It is hypothesized that patients with *T. gondii* infection have a higher risk for heart disease caused by the presence of cysts in the heart muscle. The interaction of *T. gondii* within skeletal muscle cells has been nicely described recently by (Swierzy *et al.*, 2014) who was clarified the infection of skeletal muscle cells (SkMCs) by *T. gondii*, differentiation from the highly replicative tachyzoites to dormant bradyzoites and tissue cyst formation are crucial for parasite persistence in muscle tissue. Evidence obtained in vitro and in vivo indicates that SkMCs are indeed a preferred

cell type for tissue cyst formation and long-term persistence of *T. gondii*. The differentiation of SkMCs from myoblasts to syncytial myotubes, rather than the cell type itself, is critical for parasite growth, bradyzoite formation and tissue cyst maturation. Myotube formation is accompanied by a permanent withdrawal from the cell cycle, and the negative cell cycle regulator cell division autoantigen (CDA)-1 directly or indirectly promotes *T. gondii* stage conversion in SkMCs. Moreover, host cell cycle regulators are specifically modulated in mature myotubes, but not myoblasts, following infection. Myotubes also up-regulate the expression of various pro-inflammatory cytokines and chemokines after *T. gondii* infection and they respond to IFN- γ by exerting potent anti-parasitic activity.

5.10 Estimation of some blood parameters in the Subjects in both groups Infected and non-infected with toxoplasmosis

In current study we evaluate the rate of some blood parameters in heart disease patients and controls infected and non-infected with toxoplasmosis. There was high significant difference at $p < 0.05$ observed in mean ratio of eosinophil's number between infected and not infected subjects with toxoplasmosis in both subjects. This result was coincident with other studies by Shani, (2004); Al-Ubaydi, (2011); Al-Ghezy, (2012); Al-Abudy, (2014); Al-Mosawi, (2014) and Krueger *et al.*, (2014), whereas this result was disagreed with other studies by Hakim *et al.*, (1994) and Al-Sheikh, (2004). While no significant difference observed in mean ratio of total count of leukocytes (WBCs) and haemoglobin level (Hb) between subjects infected and non-infected with anti- *Toxoplasma* antibodies. This result was agreed with other studies by Mcleod and Dowell, (2000); Al-Ubaydi, (2011); Al-Ghezy, (2012); Al-Abudy, (2014) and Al-Mosawi, (2014), while this result was different from other study by Shani, (2004).

Also there was no significant differences documented in mean ratio of basophiles, monocytes and neutrophils numbers in both infected and not infected subjects (Al-ubaydi, 2011; Al-Ghezy,2012; Al-Abudy, 2014; Al-Mosawi, 2014), while significant differences following ingestion, *T.gondii* parasites invade the gastrointestinal wall, disseminate, and elicit a strong immune response that triggers significant ancillary pathologies; eosinophilia have been reported in clinically severe cases of toxoplasmosis (MacLaren and De Souza, 2002). Eosinophils which are granulocytes involved in immune response to allergic state, some parasite infection and some autoimmune and malignant diseases. Eosinophilia (i.e. sometimes quite marked with as many as 50% of the peripheral blood white cells and substantial numbers of CSF eosinophils) can be marked in human congenital *T. gondii* infection, but the reasons for this are not known. Murine eosinophils were reported to have microbicidal capacity against antibody coated *T. gondii* (McLeod and Dowel, 2000).

Moreover, the present result showed that the mean of lymphocytes number recorded significant differences between infected and not infected heart disease patients only. This result agreed with Shani, (2004) and Al-Mosawi, (2014) who recorded there are significant differences between patients and control group in the mean percentage of total count of lymphocytes. Primary infection by *T. gondii* stimulates an effective protective immune response through production of type 1 cytokines and continuous cell-mediated immunity. After innate resistance and IFN- γ dependent mechanisms have controlled tachyzoite replication, both CD4⁺ and CD8⁺ T cells are essential to maintain bradyzoites in a latent stage. Control of acute and chronic phases of *T. gondii* infection requires both CD4⁺ and CD8⁺ T cells (Roberts *et al.*, 2007).

Conclusions and Recommendations

Conclusion

Based on the results of the present study, the following conclusions can be arrived at :

1. There are high values of anti-Toxoplasma Abs (IgG) in compare with IgM through tested by ELISA technique.
2. Estimation of cytokine levels in heart disease patients infected with toxoplasmosis award big picture about immune status in those patients.
3. Increasing of IL-12 in heart disease patients with toxoplasmosis indicate to an inflammatory status may be included in heart disease pathogenesis.
4. There is a low value of anti-inflammatory interleukin (IL-10) in patients with toxoplasmosis that indicates the opposite role of IL-10 to IL-12 in immune response against toxoplasmosis.
5. There is significant association between toxoplasmosis and heart disease patients, also there is relationship between toxoplasmosis and risk factors.

Recommendation

Through the results that have been shown in the present study, the following recommends can be arrived at :

1. Screening for anti-toxoplasma antibody in patients with heart disease is necessary to have therapy and prevent complications.
2. Further studies on the molecular level to the parasite and to identify virulence and strain type.
3. Make more studies about other cytokines and explain how they affect the toxoplasmosis and heart disease.
4. Evaluate the cell mediated immunity at those patients to understanding the exact immune status and its relation with toxoplasmosis.
5. Further studies with large number of patients with heart disease and with another tests such as histopathology.
6. Further studies about type and duration of suppressive therapy and who the effect on toxoplasmosis in heart disease patients.

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الخلاصة

داء المقوسات هو مصطلح عام للعدوى والمرض في الإنسان والحيوان يسببه طفيلي يعرف بالمقوسة الكوندية، وهذا المرض ينتشر بين فئات مختلفة من الناس، مرضى القلب واحده من هذه الفئات. بسبب حالة السكون للطفيلي، هناك غموض وأختلاف في الحالة المناعية للمرضى. هدفت هذه الدراسة للتحقيق في الحالة المناعية لمرضى القلب المصابين بداء المقوسات، و للكشف عن العلاقة المحتملة بين مرضى القلب و الطفيلي،، والتحقيق في العلاقة بين داء المقوسات، وعوامل مختلفة تشمل الجنس، العمر، مجاميع الدم، الملامسة مع القطط، ومناطق سكن المرضى.

جمعت مائة وخمسون عينة دم في الفترة الممتدة من آب عام ٢٠١٦ حتى تشرين الثاني عام ٢٠١٦ من مرضى القلب، الزائرين والراقدين في مركز القلب الطبي في محافظة ذي قار. قسمت عينات المرضى حسب نوع الجنس، الى (٨١ ذكور و ٦٩ أناث) وبأعمار تتراوح ما بين ١٨ و ٨٢ عاماً. كما جمعت مائة عينة من الدم من الأشخاص الأصحاء، وقسمت العينات كذلك حسب الجنس الى (٥٥ ذكور و ٤٥ أناث) وبأعمار تتراوح ما بين ١٩ و ٥٦ عاماً. تم اختبار العينات من كل من مرضى القلب والسيطرة (الأصحاء) بأستعمال تقنية اللأليزا (تقنية الانزيم المرتبط) للكشف عن الأجسام المضادة لطفيلي التوكسوبلازما ، كذلك تم اختبار العينات الموجبة للتوكسوبلازما لتحديد مستويات الأنترلوكينات (IL-١٠، IL-١٢ و IL-١٧). أظهرت النتائج ان النسبة الكلية لانتشار الأجسام المضادة لطفيلي التوكسوبلازما في مرضى القلب هي ٤٤% وهي اعلى من النسبة التي ظهرت في الأصحاء ١٩%، مع وجود فرق معنوي واضح بين المجموعتين. كذلك هنالك فروق معنوية واضحة بين نسبة إصابة الذكور و الأناث في الأصحاء، حيث كانت نسبة الإصابة في الذكور أعلى منها في الأناث. وجود الأجسام المضادة لداء المقوسات في كل أنواع مرضى القلب مع اختلاف كبير فيما بينها، وأعلى نسبة كانت ٥٥.٨% في مرضى تضيق الصمام التاجي ، وأقل نسبة كانت ٣٠.٣% في مرضى احتشاء عضلة القلب. هناك اختلافات كبيرة بين الفئات العمرية في نسبة الإصابة ، حيث أن نسبة الإصابة بالطفيلي في مرضى القلب كانت أعلى قيمة هي (٦٣.٢ %) في الفئة العمرية (٦١-٧٥) سنة، وكان أدنى قيمة هي (٢٦.٣ %) في الفئة العمرية (١٥-٣٠) سنة.

أما في الأصحاء المصابين بالطفيلي لا توجد اختلافات كبيرة بين الفئات العمرية وداء المقوسات حيث ظهرت أعلى نسبة للأصابة هي (١٩.٥ %) في الفئة العمرية (٣١-٤٥) سنة، و أدنى نسبة هي (٨.٦ %) في الفئة العمرية (١٥-٣٠) سنة.

فيما يخص مجاميع الدم أعلى نسبة للأصابة ظهرت في الأشخاص ذات فصيلة دم AB+ في كل من مرضى القلب والأصحاء. أظهرت النتائج أن معدل الانتشار لطفيلي داء المقوسات ارتبط إلى حد كبير بالتلامس المباشر مع القطط. أما فيما يخص التوزيع الجغرافي للمرضى كانت النسبة المئوية لمعدل انتشار الطفيلي في المناطق الريفية ٥٢.٥ % بين مرضى القلب و ٣٢ % بين الأصحاء أكثر من نسبة الأصابة في المناطق الحضرية المأهولة بالسكان في كل من مرضى القلب والأصحاء (34.3% و 6%) على التوالي.

في الدراسة الحالية تم قياس بعض معايير الدم في كل من مرضى القلب والأصحاء المصابين وغير المصابين بالطفيلي وكانت هنالك فروقات معنوية واضحة عند $p \leq 0.05$ في نسبة خلايا الحمضة (eosinophil) في كل من المرضى والأصحاء المصابين بالطفيلي عند مقارنتهم مع غير المصابين. في حين لم يظهر فرق معنوي في نسبة العدد الكلي للكريات البيضاء، مستوى الهيموغلوبين (خضاب الدم)، خلايا العدلة، القعدة والخلايا الوحيدة في كل المصابين وغير المصابين من مرضى القلب والأصحاء. وعلاوة على ذلك، سجلت متوسط عدد الخلايا الليمفاوية اختلافات كبيرة بين المصابين وغير المصابين من مرضى القلب فقط. أما نتائج اختبار الأنترلوكينات أظهرت ارتفاع مستوى الأنترلوكين (IL-12 = ٨٢.٨٥ pg/ml) في مرضى القلب المصابين بطفيلي التوكسوبلازما عند مقارنتها مع عينات السيطرة الأنترلوكين (IL12=60.97 pg/ml) ، بينما لا توجد اختلافات واضحة في مستوى الأنترلوكين IL-١٠ بين مرضى القلب وعينات السيطرة ، كذلك أظهرت النتائج انخفاض في مستوى الأنترلوكين ١٧ في مرضى القلب. زيادة مستوى الأنترلوكين ١٢ في مرضى القلب المصابين بداء المقوسات يشير الى نشاط المناعة الخلوية في مرضى القلب ، ويمكن أن يؤدي إلى إثارة حالة التهاب في صمامات القلب والعضلات ثم أمراض القلب الأكثر شدة والحاجة للمعالجة المعقدة



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دراسة مصلية ومناعية لداء المقوسات في مرضى القلب

رسالة مقدمة الى

مجلس كلية العلوم – جامعة ذي قار
وهي جزء من متطلبات نيل شهادة الماجستير في علوم الحياة

من قبل

حيدر صباح عبد الحسين

بكالوريوس علوم حياة /جامعة ذي قار

٢٠١١

إشراف

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