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ESTIMATION OF SERUM LEVELS OF HUMAN INTERLEUKIN-12 IN IRAQI WOMEN WITH TOXOPLASMOSIS

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ESTIMATION OF SERUM LEVELS OF HUMAN INTERLEUKIN-12 IN IRAQI WOMEN WITH TOXOPLASMOSIS

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May 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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Saja Idan Neamah KINANAH

ABSTRACT

ESTIMATION OF SERUM LEVELS OF HUMAN INTERLEUKIN-12 IN IRAQI WOMEN WITH TOXOPLASMOSIS

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Master of Science in Biology

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May 2023

Toxoplasmosis is a significant disease due to infection by *Toxoplasma gondii* that can be obtained postnatally by ingesting contaminated food or water or through sexual transmission. The oocyst is discharged in the faeces of the definitive host. The cat is the host the agent and can cause to infection. The infection can come from the consumption of meat from an organism (intermediate host) that is chronically infected with toxoplasmosis. *T. gondii* is capable of infecting nearly every species of a warm-blooded animal. An acute infection can be identified by the proliferation of tachyzoites, which develop quickly but have the potential to encyst into bradyzoites, which grow more slowly but can live in the host for their whole lifetime, driving to infection into a chronic state. It is estimated that nearly one-fourth of the population in the United States is infected with *T. gondii*. It is believed that as many as one billion people worldwide live with a persistent infection. In this case-control study, which was conducted between September 2022 and March 2022 at Al-Imameen Al-Kazimin Teaching Hospital, there were 180 volunteers involved. About 90 were healthy and made up the standard study group. The other 90 people had been clinically diagnosed with toxoplasmosis. To participate in the research, participants gave their verbal permission. Direct interviews are conducted with each patient to collect the necessary information. Inclusion criteria: Toxoplasmosis patients aged 20-50 years. Exclusion criteria: Toxoplasmosis patients aged > 50 and < 20 years with other inflammatory diseases. In the healthy females versus females with toxoplasmosis, the final results of interleukin 12, IgM, and IgG of patients and controls were (719.08 ± 20.02 and 12.41 ± 0.84 pg/ml), (0.39 ± 0.01 and 0.16 ± 0.01

IU/ml), and (721.48 ± 23.35 and 16.01 ± 0.66 IU/ml) respectively, it showed a significant difference between healthy females versus females with toxoplasmosis ($p \leq 0.001$) for each variable separately, Age of patients and controls was (28.63 ± 7.94 and 28.03 ± 6.74 years) respectively, there is no relationship between age and severity of toxoplasmosis infection ($p \leq 0.585$). In conclusion, the severity of toxoplasmosis in females is correlated with serum interleukin-12 levels. Interleukin-12 levels in Toxoplasmosis-infected women can assess infection severity. The severity of toxoplasmosis in females is directly related to their serum IgM and IgG levels. It is observed that the age does not effect toxoplasmosis severity.

2023, 47 pages

Keywords: Toxoplasmosis, *Toxoplasma gondii*, Interleukin 12, IgM, IgG

ÖZET

TOKSOPLAZMOZLU IRAKLI KADINLARDA İNSAN İNTERLÖKİN-12 SERUM DÜZEYLERİNİN TAHMİNİ

Saja Idan Neamah KINANAH
Biyoloji, Yüksek Lisans
Tez Danışmanı: Dr. Öğr. Üyesi Serdal TARHANE
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Toksoplazmoz, doğumdan sonra kontamine yiyecek veya suların alınması veya cinsel yolla bulaşabilen *Toxoplasma gondii* enfeksiyonuna bağlı önemli bir hastalıktır. Ookist, kesin konağın dışkısıyla atılır. Kediler bu etkene ev sahipliği yaparak enfeksiyona yol açabilir. Enfeksiyon, toksoplazmoz ile kronik olarak enfekte olan bir organizmanın (ara konakçı) et tüketiminden kaynaklanabilir. *T. gondii* sıcakkanlı hayvanların hemen hemen her türünü enfekte edebilir. Akut bir enfeksiyon, hızla gelişen ancak bradizoitlere dönüşme potansiyeline sahip olan ve daha yavaş büyüyen ancak tüm yaşamları boyunca konakçıda yaşayabilen ve enfeksiyonu kronik bir duruma sürükleyen takizoitlerin çoğalmasıyla tanımlanabilir. Amerika Birleşik Devletleri'ndeki nüfusun yaklaşık dörtte birinin *T. gondii* ile enfekte olduğu tahmin edilmektedir. Dünya çapında bir milyar kadar insanın kalıcı bir enfeksiyonla yaşadığına inanılmaktadır. Al-Imameen Al-Kazimin Eğitim Hastanesinde Eylül 2022 ile Mart 2022 arasında yürütülen bu vaka kontrol çalışmasına 180 gönüllü dahil edildi. Yaklaşık 90 sağlıklı kişi standart çalışma grubunu oluşturdu. Diğer 90 kişiye klinik olarak toksoplazmoz teşhisi konuldu. Araştırmaya katılmak için katılımcılardan sözlü izin alındı. Gerekli bilgileri toplamak için her hastayla doğrudan görüşmeler yapıldı. Gönüllülerin dahil edilme kriterleri: 20-50 yaş arası toksoplazmoz hastası olmaları. Hariç tutulma kriterleri: Diğer enflamatuar hastalıkları olan > 50 ve < 20 yaşındaki toksoplazmoz hastası olmaları olarak belirlendi. Toksoplazmozlu kadınlara karşı sağlıklı kadınlarda, hasta ve kontrollerin nihai interlökin 12, IgM ve IgG seviyeleri (719,08 ± 20,02 ve 12,41 ± 0,84 pg/ml), (0,39 ± 0,01 ve 0,16 ± 0,01 IU/ml) ve (sırasıyla 721,48 ± 23,35 ve 16,01 ± 0,66 IU/ml) olarak belirlendi.

Değerler, sağlıklı kadınlar ile toksoplazmozlu kadınlar arasında her bir değişken için ayrı ayrı anlamlı bir fark gösterdi ($p \leq 0,001$). Hastaların ve kontrollerin yaşları sırasıyla ($28,63 \pm 7,94$ ve $28,03 \pm 6,74$) idi, yaş ile toksoplazmoz enfeksiyonunun şiddeti arasında bir ilişki bulunamadı ($p \leq 0,585$). Sonuç olarak, kadınlarda toksoplazmozis şiddeti serum interlökin-12 seviyeleri ile ilişkilidir. Toksoplazmoz ile enfekte olmuş kadınlarda interlökin-12 seviyeleri enfeksiyonun şiddetini değerlendirebilir. Dişilerde toksoplazmozun ciddiyeti doğrudan serum IgM ve IgG seviyeleri ile ilişkilidir. Yaşın toksoplazmoz şiddetini etkilemediği gözlemlendi.

2023, 47 sayfa

Anahtar Kelimeler: Toksoplazmoz, *Toxoplasma gondii*, İnterlökin 12, IgM, IgG

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Saja Idan Neamah KINANAH

Çankırı-2023

CONTENTS

LIST OF SYMBOLS

IU	International units
μL	Microliter
mL	Milliliter
nm	Nanometer
NS	Non-significant
pg	Picogram
rpm	Revolutions per minute

LIST OF ABBREVIATIONS

APC	Antigen presenting cell
TgMYO	Calcium binding proteins
CD4+	Cluster of differentiation 4+
CD8+	Cluster of differentiation 8+
DCs	Dendritic cells
ELISA	Enzyme-linked immunosorbent assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IFN	Interferon
IL-12	Interleukin 12
IL-23	Interleukin 23
IL-27	Interleukin 27
MICs	Micronemal proteins
RONs	Proteins associated with host cell invasion
TNF	Tumor necrosis factor

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Toxoplasmosis is a significant disease due to infection by *Toxoplasma gondii* that can be obtained postnatally by ingesting contaminated food or water or through sexual transmission. The oocyst is discharged in the faeces of the definitive host. The cat is the quixotic host and can guide to infection (Attias *et al.* 2020). The infection can come from the consumption of meat from an organism (intermediate host) that is chronically infected with toxoplasmosis. *T. gondii* is capable of infecting nearly every species of a warm-blooded animal. An acute infection can be identified by the proliferation of tachyzoites, which develop quickly but have the potential to encyst into bradyzoites, which grow more slowly but can live in the host for their whole lifetime, driving to infection into a chronic state. It is estimated that nearly one-fourth of the population in the United States is infected with T. gondii. It is believed that as many as one billion people worldwide live with a persistent infection (Robben *et al.* 2004).

In immunocompetent persons, acute toxoplasmosis can sometimes be passed without any symptoms, and when it does cause symptoms, they are typically minor and resemble those of the flu. However, severe signs, such as retinochoroiditis, pneumonitis, and encephalitis, can appear during acute infections. These symptoms can be life-threatening. Toxoplasmosis, which affects immunocompromised individuals, frequently causes reactivation of chronic illnesses, which, if neglected, results in encephalitis, which is always fatal (Robben *et al.* 2004). In healthy persons, the progression of the infection is characterized by the acute phase linked with the dispersion of fastly dividing tachyzoites that can infect almost all cells. This phase is connected with the acute phase of the infection. After this, a long-term chronic phase will begin, that will coincide with an increase in adaptive immunity of host. Therefore, the state of the immune system of the host plays a significant role in the toxoplasmosis pathophysiology (Quan *et al.* 2013).

It has been demonstrated that members of the interleukin-12 cytokines family play important regulatory roles in both the adaptive and innate immune response. The interleukin-12 is the subject of a significant amount of research, relatively slight is

understood about interleukin-27 and interleukin-23. Interleukin -12 is a heterodimer in terms of its three-dimensional structure, its two subunits, p40 and p35, are identical to one another, but the p40 subunit is shared by both subunits. In addition to this, the IL-12R1 chain is shared by their heterodimeric receptors. The presence of *T. gondii* triggers the release of a number of cytokines from macrophages. These cytokines include TNF, IL-23, and IL-12. Because IL-12 plays such an important part in the process of inducing IFN- production, it is necessary for protection against both acute and chronic forms of toxoplasmosis (Lieberman *et al.* 2004). In response to antigens of *T. gondii*, macrophages, neutrophils, and dendritic cells all begin producing IL-12 early on. Even if the IL-12 absence can only supply a bounded method of resistance to toxoplasmosis, in the resistance mechanism against this infection, the IL-12 can play the primary role. As a result, IL-12 plays roles in innate immunity that are distinct from one another yet complementary to one another (Quan *et al.* 2013).

1.1 The Study Objectives

1. Evaluation of serum levels of human interleukin-12 in Iraqi women with toxoplasmosis.
2. Study the possibility of using serum levels of human interleukin-12 as biomarkers in the early diagnosis of Toxoplasmosis.
3. Using of using serum levels of human interleukin-12 as biomarkers in Toxoplasmosis development monitoring.
4. Explanation of influence of the consequences of Toxoplasmosis on serum levels of human interleukin-12.

2. LITERATURE REVIEW

2.1 Toxoplasmosis

Toxoplasmosis is a parasitic disease pushed by a protozoan parasite named "*T. gondii*" that is highly prevalent everywhere the globe. The parasite *T. gondii* is responsible for the transmission of toxoplasmosis (Attias *et al.* 2020). However, even though most infected people are without symptoms or only exhibit moderate symptoms, if the infection occurs during pregnancy, it can generate severe neurological harm and death to the unborn child if not treated as soon as it appears. When it comes to immunodeficient individuals, it can be a life-threatening condition (Ehmen and Lüder, 2019). Its life cycle is complicated because *T. gondii* has more than one infectious form and several different transmission pathways (Attias *et al.* 2020).

2.1.1 *Toxoplasma gondii* classification

The *T. gondii* classification is often based on a formal comparison among the parasite's different life stages, the most important of which is a sexual phase, a coccidian spherical parasite belonging to the phylum Apicomplexa, an ancient group that includes 5000 species of primary parasites. Spheroids were classified in the family Eimeridae, and after the life cycle of the *T. gondii* discovery, they were placed by other classifiers in the families Sarcocystide, Eimeridae and Toxoplasmatidae, The *T. gondii* classification as in follwing sequence, "Phylum (Apicomplexa), Class (Sporozoa), Subclass (Coccidiansina), Order (Eimeriorina), Family (Toxoplasmatidae), Genus (*Toxoplasma*), Species (*gondii*)" (Schoch *et al.* 2020).

2.1.2 Parasite stages

There are three infective stages of the Toxoplasma parasite that are important for understanding and diagnosing the disease. These stages are trophozoite, which includes the fast-reproducing intracellular tachyzoite that resides within the pseudocyst, and the

slow-reproducing phase of bradyzoite, which resides within tissue cysts. This sporozoite stage lives inside the oocyst that is environmentally resistant (Attias *et al.* 2020).

Oocyst:

Subspherical to spherical, the unsporulated oocysts are ten by twelve nm in diameter. The two colourless layers surrounding the oocyst wall that are visible under a light microscope. The oocyst lacks polar granules, and the sporont is nearly full. With proper aeration and temperature, sputum can be produced outside the cat in about one to five days after expulsion has occurred (Dubey *et al.* 1998).

They have an 11-13 nm diameter and are subspherical to elliptical in shape. There are two elliptical, non-Stieda-bodies in each of the two individual oocyst and 6 by 8 nm sporocysts are the standard size. In this case, there is only one sporocyst residuum, and there is no one else. Four sporozoites are seen in each sporocyst. There is a big nucleus with amorphous nucleoplasm in the cytoplasm of an unsporulated oocyst (zygote). In the zygote, there are few micropores in the unit membrane. During the second division of the nucleus, a second limiting membrane is created, which results in the formation of four nuclei along the zygote's perimeter. Each of the two nuclei in each of the resulting spherical sporoblasts is generated when the cytoplasm divides (Dubey *et al.* 1998).

The sporoblasts become longer and sporocysts are generated as the sporulation progresses. There are two sporoblast membranes that form the sporocyst wall, and the cytoplasmic mass's plasmalemma forms the inner layer of this wall. The deepest layer of the sporocyst is made up of four bent plates. The sporocyst wall has a dip at the junction where four liplike sutures (explained below) connect the plates. Sporozoite production begins when two dense plaques (anlagen) develop at the ends of the sporocyst. Sporozoite anlagen are formed by the division of each nucleus into two halves. Each sporocyst produces four sporozoites. Immediately after sporozoites are created, there is a large, single-unit membrane around the remaining body (Dubey *et al.* 1998).

Sporulated oocyst walls have an electron-dense interior layer, an electron-fluid middle layer, and an electron-dense outer layer. When the oocyst wall was forming, two membranes were put down between the outer and inner layers, and these vestiges are found in the middle layer of the wall today. The outer layer of oocysts can be removed by treating them with 1.3 per cent sodium hypochlorite (Clorox). When it comes to oocyst walls, one micropyle may be seen in the middle of each one. An indentation with a diameter of 350 nm, the micropyle is composed of three layers that run parallel to the three layers that make up the oocyst wall. Micropyles have a thin, moderately electron-dense outer layer and an electron-dense, disk-shaped inner layer that is thicker than the inner layer of oocytes' inner wall. Even though the micropyle's role in the oocyst wall has not been established, it may represent a permeable region that is vulnerable to the effects of CO_2 and enzymes which permit the bile salts access and trypsin, that induce sporozoite exocytosis into the oocyst (Dubey *et al.* 1998).

Tachyzoite or pseudocyst:

The word "tachyzoites" (tachos = speed in Greek) was used by Frenkel to describe any cell of the intermediate host and non-intentional epithelium of the definitive host that rapidly multiplied. It has been replaced by the term "tachyzoite," which means "feeding" in Greek. Alternatively, the term "tachyzoites" might refer to an endodyozoite or an endozoite. Groupings of tachyzoites, clones, or terminal colonies are all terms used to describe tachyzoites in large groups (Dubey *et al.* 1998).

There are two distinct shapes of tachyzoite: the front (conoidal) end, which is pointy, and the posterior (rounded). Various organelles and inclusion bodies are found in the tachyzoite's ultrastructure, including an apical rings, pellicle, conoids, polar rings, rhoptries, micropores, micronemes, subpellicular microtubules, mitochondrions, the endoplasmic reticulum, ribosomes, the golgi complex, smooth and rough endoplasmic reticula. When cells divide into two halves, each half has a nucleus and chromatin, which are positioned in the center of each half, respectively (Dubey *et al.* 1998).

There are no cilia, flagella, or pseudopodia visible to the naked eye in tachyzoites, despite their ability to gliding, undulating and rotating. Despite the fact that their exact roles in parasite growth and development are still being debated, it is likely that the conoid, rhoptries, micropores, and micronemes play a role in this. Before penetration, the parasite explores the host cell plasmalemma, the conoid can spin, tilt, stretch, and retract. Rhoptries secrete their contents to the outside through the plasmalemma right above the conoid as part of their host cell penetration function. They have an enzyme that breaks down proteins. The pellicle's outer membrane invaginates the micropore, resulting in a cytosome-like structure (Dubey *et al.* 1998).

Bradyzoite:

The name "bradyzoite" (brady = slow in Greek) to characterize an organism that grows slowly within a tissue cyst. Bradyzoites, or cystozoites, are a type of bacterium (Dubey *et al.* 1998).

The bradyzoites divide via endodyogeny, creating tissue cysts that develop and remain intracellular (Attias *et al.* 2020, Dubey, 2009). Depending on their age, tissue cysts can contain hundreds of bradyzoites despite being only five nm in diameter and containing just two bradyzoites at their youngest. Intramuscular cysts, on the other hand, tend to be long and elongated, with a diameter of at least 70 nm. Even though tissue cysts can form in visceral organs, such as the kidneys and lungs, they are more common in the brain, eyes and skeletal and cardiac muscle tissue. It is likely that undamaged tissue cysts do not do any damage and can remain in the host's body for the rest of their lives without eliciting any host inflammatory reaction (Dubey *et al.* 1998).

Each crescent-shaped bradyzoite is approximately 7 by 1.5 nm and is contained within a thin, elastic cyst wall. The cytoplasm of the host cell is where the tissue cyst forms. A variety of silver impregnation methods have produced varying results when applied to cyst walls with argyrophilic cyst walls. Bodian protoargol and Palmgren silver both leave dark stains on the cyst wall, whereas methenamine silver does not. Lack of methenamine silver staining suggests that glycogen and other polysaccharides are absent from the cyst

wall. Components of a cyst's wall are derived from the *T. gondii* and from the host cell (Dubey, 2009). A granular material fills the area between the bradyzoites in the final product. Older tissue cysts tend to have degenerating bradyzoites. The cyst wall has only a modest PAS positive signal (Dubey *et al.* 1998).

2.2 Life cycle

Toxoplasma gondii is an infectious parasite responsible for toxoplasmosis, a zoonosis disease origin that is of great medical concern and can be passed on in a few different ways. During the last few decades, significant strides have been made in managing various infectious diseases generated by parasitic protozoa, particularly those that carry out a portion of their life cycle inside the host's cells. Despite this, there are still many obstacles to overcome, including the containment of the epidemic and the creation of novel chemotherapeutic drugs with a low risk of side effects but a high degree of specificity. As is the case with the Chagas disease, certain regions of the world are more likely to be affected by certain conditions than others. Other diseases, such as toxoplasmosis, are found in a widespread manner worldwide (Robert-Gangneux and Dardé, 2012).

2.2.2 Pathways of transmission

It is common knowledge that *T. gondii* may infect various hosts, including birds, terrestrial animals, and aquatic mammals. Because only the asexual stages of the parasite develop in these animals, we classify them as intermediate hosts Figure (2.1) (Attias *et al.* 2020). Only members of the "family Felidae", such as a domestic cat, can go through the stages of sexual development. As a result, we can confidently refer to them as authoritative hosts (Dubey, 2009).

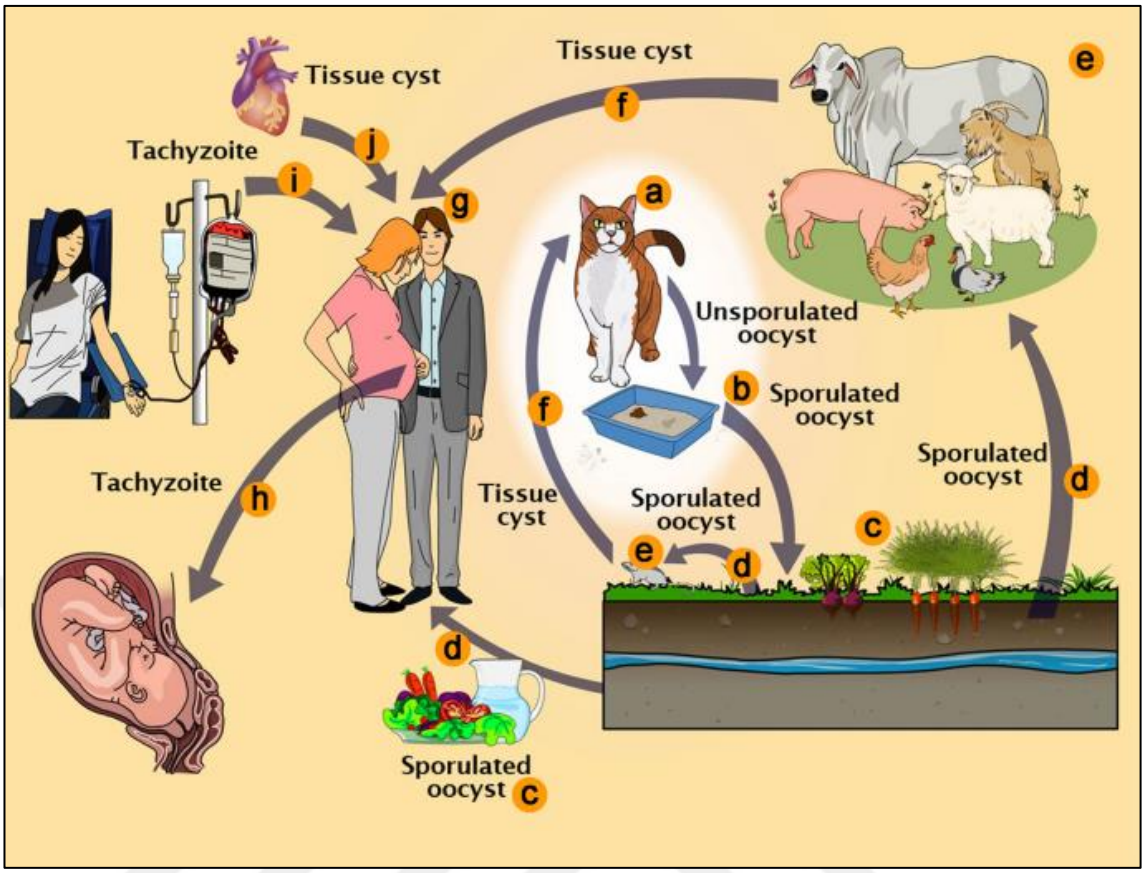

Figure 2.1 Transmission pathways of *Toxoplasma gondii* (Attias *et al.* 2020)

2.2.3 Life cycle in the final host

Only members of the Felidae family are capable of carrying out the sexual cycle of the *Toxoplasma gondii* parasite. The vast majority of research pertaining to this stage of the cycle has been conducted on cats, and more specifically on young kittens (Dubey, 2009). There are not that many articles that deal with the *T. gondii* cycle in this host since it is difficult to keep cats in the laboratory and sacrifice them. Ingestion of prey that contains tissue cysts or oocysts that contain sporozoites that have been discharged by another feline is the major mode of transmission of the illness from one feline to another (Figure 2.2) (Attias *et al.* 2020). The cysts wall is broken down after ingestion in the stomach, most likely due to the proteolytic enzyme activity and the low pH. This results in the release of either bradyzoites or sporozoites, depending on the parasite. the first cells to be infected are intestinal epithelial cells and will transform into an asexual reproduction stage that is

characterized by the existence of many nuclei called schizonts (Attias *et al.* 2020). Schizonts can be distinguished from other stages of the life cycle by their size as presented in Figure (2.2).

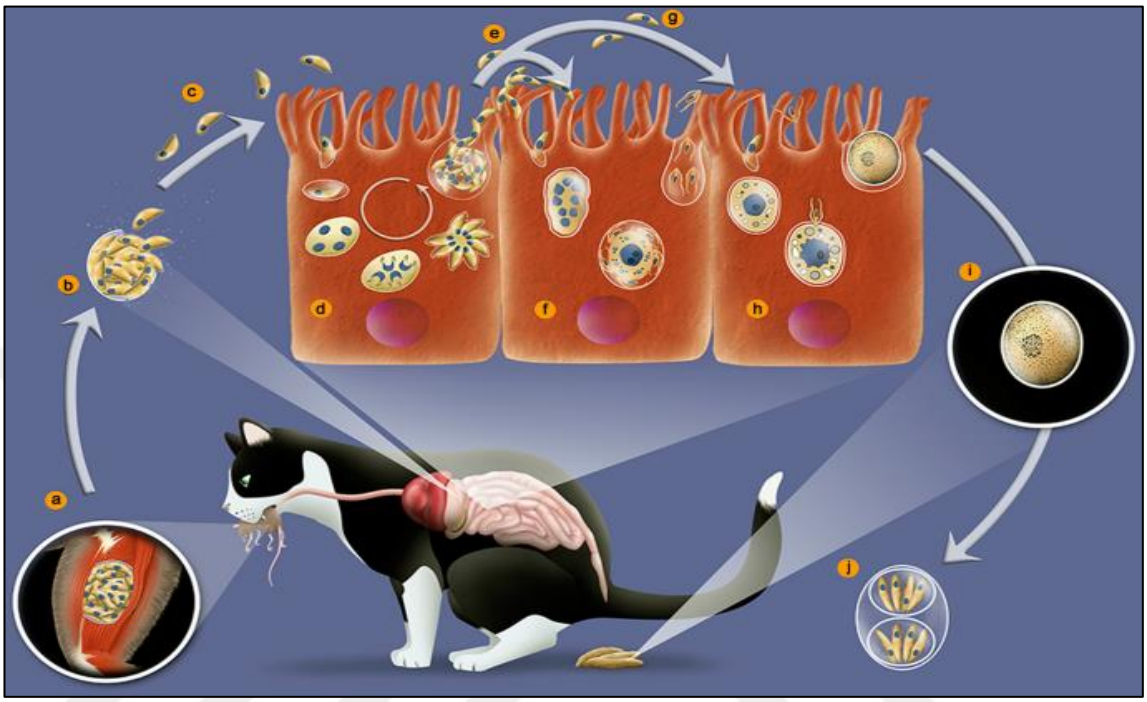

Figure 2.2 Life cycle of *T. gondii* in cats (Attias *et al.* 2020)

In schizogony, cell individualization comes after nuclear divisions. After nuclear division, the complex of the inner membrane for individualizing each merozoite forms in similarity with the apical complex, which includes rhoptries and micronemes. Around each nucleus, these cytoplasmic structures form daughter cells, termed merozoites. Enterocyte rupture produces several merozoites, which can infect novel enterocytes and then divide by schizogony. Per cycle of schizogonic produces numerous merozoites that rapidly penetrate fresh enterocytes, exponentially increasing parasite numbers. *T. gondii* schizonts have been identified in feline intestinal epithelial cells (Attias *et al.* 2020).

2.2.4 The life-cycle in the intermediate host

A few different routes can lead to infection of intermediate hosts. The walls of both oocysts and tissue cysts are broken down via digestive enzymes, which results in the release of sporozoites or bradyzoites, which, once inside the novel host, travel through the body via a mechanism that is exclusive to the gliding (Frénal *et al.* 2017, Opitz and Soldati, 2002). First proteins secreted by protozoa are micronemal proteins, which play a critical role in gliding and early attachment to host cells. the actin-myosin motor is located between the inner pellicle and plasma membrane of the cell, and this is where proteins attached to it work together to allow it to glide (Attias *et al.* 2020). Tachyzoite plasma membranes contain microneme proteins (MIC2 and AMA1) that have been incorporated into the glide. The receptors on the plasma membrane of the host cell are recognized and attached by these microneme proteins. MIC 2 and AM1 are linked to an aldolase that is bound to filamentous actin that is driven by a TgMYO myosin motor and several GAP proteins (GAP40, GAP50, GAP45, and GAPM) that connect the three membranes of the alveoli network and the pellicle (Kato, 2018). While moving forward, the tachyzoite is propelled by this convoluted arrangement toward a new host cell (Attias *et al.* 2020).

T. gondii first attaches itself to the host cell's surface and then moves the apical side sequentially. Proteins from apical organelles, such as rhoptries and micronemes, are released and internalized as a result of this reorientation. It must first form "moving junctions" with the plasma membrane in order to invade the host cell. This moving junction allows tachyzoites to enter host cells, forming a circular ring around them. This procedure allows the micronemal protein AMA1 to attach to the rhoptry protein RON 2 before being inserted, secreted, and exposed to the membrane of the host cell *(Attias et al.* 2020).

2.3 Epidemiology

"*Toxoplasma gondii*" is a parasite that can be found in mammals as well as birds (Attias *et al.* 2020). Even though cats are the only definite host and, as a result, the only source of infectious oocysts, other animals and birds are nonetheless capable of developing tissue

cysts. In contrast to feline illnesses, which are often asymptomatic, human pregnancies during which an infection is present might result in severe disease in the developing fetus (Elmore *et al.* 2010). The owners of cats can lower the danger of infection to their pets by ensuring that all of their cats are kept indoors and by avoiding feeding them raw meat. Infection of humans typically occurs through the eating of oocysts that have been contaminated in the soil or water, or through the consumption of tissue cysts that have been undercooked in meat. Direct contact with cats is not believed to be a primary risk for human infection due to the fact that cats are very clean animals, that they shed non-infectious oocysts, and that oocyst shedding only lasts for a brief period of time (Elmore *et al.* 2010).

2.3.2 *Toxoplasma gondii* infection between rural and urban areas

Toxoplasma gondii infection, commonly known as toxoplasmosis, can exhibit variations in prevalence and risk factors between rural and urban areas. While there is limited specific information comparing infection rates in rural and urban settings, we can provide a general overview of the factors that might influence the occurrence of Toxoplasma infection in these areas (Abdul Ameer Jaber and Aamer Noori, 2021, Morais *et al.* 2021, Munoz-Zanzi *et al.* 2016).

Exposure to Intermediate Hosts:

Rural Areas: Rural regions often have a higher presence of domesticated animals, such as livestock and outdoor cats. These animals can serve as intermediate hosts for Toxoplasma, which increases the likelihood of transmission in rural areas. Agricultural practices, including proximity to farms and contact with soil, may also contribute to exposure to the parasite (Abdul Ameer Jaber and Aamer Noori, 2021, Morais *et al.* 2021, Munoz-Zanzi *et al.* 2016).

Urban Areas: Urban settings typically have a higher population density, which can result in an increased number of domestic cats. However, due to limited outdoor access or

confinement to indoor environments, the contact between cats and potentially infected intermediate hosts might be reduced. Consequently, the risk of Toxoplasma transmission in urban areas could be comparatively lower (Abdul Ameer Jaber and Aamer Noori 2021, Morais *et al.* 2021, Munoz-Zanzi *et al.* 2016).

Hygiene Practices:

Rural Areas: Hygiene practices related to waste management and animal handling may be less developed in rural areas compared to urban areas. This can increase the risk of Toxoplasma transmission through exposure to contaminated soil or water sources, especially if agricultural practices involve the use of untreated or improperly managed animal waste (Abdul Ameer Jaber and Aamer Noori 2021, Morais *et al.* 2021, Munoz-Zanzi *et al.* 2016).

Urban Areas: Urban areas generally have better waste management systems and improved hygiene practices. Proper sanitation, efficient waste disposal, and water treatment methods help minimize the risk of Toxoplasma contamination in urban environments (Abdul Ameer Jaber and Aamer Noori, 2021, Morais *et al.* 2021, Munoz-Zanzi *et al.* 2016).

Dietary Habits:

Rural Areas: Traditional dietary habits in rural areas may involve the consumption of raw or undercooked meat, including meat from livestock potentially infected with Toxoplasma. This can increase the risk of acquiring the parasite through foodborne transmission (Abdul Ameer Jaber and Aamer Noori, 2021, Morais *et al.* 2021, Munoz-Zanzi *et al.* 2016).

Urban Areas: Urban populations often have access to a wider range of food options, including properly cooked meat. Additionally, urban areas may have stricter food safety regulations and cultural practices that promote safer food handling and cooking practices.

These factors can help reduce the risk of Toxoplasma infection through the consumption of contaminated food (Abdul Ameer Jaber and Aamer Noori, 2021, Morais *et al.* 2021, Munoz-Zanzi *et al.* 2016).

Healthcare Access and Awareness:

Rural Areas: Limited access to healthcare facilities, diagnostic testing, and treatment options can hinder the identification and management of Toxoplasma infections in rural areas. Furthermore, the level of awareness about the disease and its prevention may be relatively lower among the rural population (Abdul Ameer Jaber and Aamer Noori, 2021, Morais *et al.* 2021, Munoz-Zanzi *et al.* 2016).

Urban Areas: Urban populations typically have better access to healthcare services, including diagnostic tools and treatment options for Toxoplasma infection. Higher levels of awareness about the disease, facilitated by more extensive health education campaigns, may also lead to more proactive prevention measures in urban areas (Abdul Ameer Jaber and Aamer Noori, 2021, Morais *et al.* 2021, Munoz-Zanzi *et al.* 2016).

While these comparisons provide a general understanding, it is important to recognize that specific infection rates and risk factors can vary within rural and urban areas. Factors such as geographical location, socioeconomic conditions, and cultural practices can influence the prevalence and transmission dynamics of Toxoplasma infection. Therefore, to obtain more accurate and up-to-date information, it is recommended to refer to studies or reports conducted in specific regions or countries of interest (Abdul Ameer Jaber and Aamer Noori, 2021, Morais *et al.* 2021, Munoz-Zanzi *et al.* 2016).

2.4 Pathogenicity

In healthy people, *T. gondii* rarely causes major illness, but the parasite's great frequency makes it an important zoonotic pathogen. An equilibrium between acute and chronic disease is expected to favor parasite transmission since parasite and intermediate host

coevolution has taken place. Because of the domestication of cats and the subsequent spread of *T. gondii*, it is believed that the parasite strains that infect people in the Old World were selected by the domestic cat and mouse transmission cycle that emerged around 11 thousand years ago. Humans, on the other hand, may be regarded a dead end for *T. gondii* transmission to the final host because they are an uncommon or unavailable prey item for felids. While the parasite can circumvent its reproductive cycle in middle hosts felids cannot hunt on by feeding on or transmitting vertically to the progeny, this reduces genetic diversity may perpetuate the clonal population pattern. Because of this, illnesses in Europe may be attributed to strains that are rare, homogenous, and moderately virulent. South American strains, on the other hand, have a larger range of pathogenicity and variety, and these strains certainly contain more aggressive variations. In individuals who are not particularly immunocompromised, these strains can cause significant harm and even death, possibly due to the parasite's inability to adapt to the host's environment. That the delicate equilibrium between parasite virulence and host adaptability for parasite persistence might be unstable is brought home to us by this discovery (Sanchez and Besteiro, 2021).

2.5 The immunostimulation against *T. gondii*

Strong Th1 cell-mediated responses and high levels of IFN- are required in the murine system to control infection at both the acute and chronic stages of the immune response against *T. gondii* (Suzuki *et al.* 1988, Yap and Sher, 1999). In order to protect against both acute and chronic toxoplasmosis, IL-12 plays an important role in inducing the production of IFN (Robben *et al.* 2004). First, dendritic cells (DCs)4, neutrophils and macrophages produce IL-12 in response to *T. gondii* antigens. Myeloid differentiation factor 88, a key adaptor protein in the Toll-like receptor (TLR) signaling pathway, is required for the induction of interleukin-12 (IL-12) in response to antigens, suggesting that innate immunity plays a significant role in the control of infection (Robben *et al.* 2004).

Different varieties of *T. gondii* strains have been shown to exhibit varying degrees of pathogenicity in mice. The quick formation of a large parasite burden in the host is a

prominent hallmark of deadly infections caused by type I strains. This finding hints that processes that take place early on in the infection may be responsible for setting the stage for the eventual outcome. The purpose of this study is to investigate the early interaction that takes place between uninfected APC and living *T. gondii* (Robben *et al.* 2004).

2.5.2 Interleukin-12

Interleukin-12 (IL-12) is a heterodimeric cytokine that belongs to a wider family of proteins known as the IL-12 related cytokines (Walter, 2006). It possesses broad immunoregulatory capabilities and has a heterodimeric structure. The IL-12-related cytokines are produced by five genes that are separately controlled, and these genes can either homodimerize or heterodimerize to yield seven different members of the secreted family. This essay is going to focus mainly on IL-12, p40, and p80, despite the fact that these proteins all have overlapping as well as separate biological features. An immune cell stimulant known as IL-12, which is a disulfide-linked heterodimer made of a p40 and p35 subunit, has been discovered. It encourages the differentiation and proliferation of T cells and boosts the production of interferon-gamma (Walter, 2006). Multiple immunologic processes, including CD8+ and CD4+ T-cell differentiation, host defense and autoimmunity, tumor cell recognition, alloimmunity and host response to vaccine administration have been found to be controlled by IL-12, according to human studies and murine experimental models. There are two distinct functions for the p80 homodimer. One is to compete with and attract macrophages, while the other is to act as an inhibitor of IL-12. Some of the specific topics covered in this article include the structure, regulation, function and involvement of IL-12-related cytokines in respiratory disease. Receptors and their importance are also discussed (Walter, 2006).

2.5.3 Interleukin-12 secretion in response to infection with *T. gondii*

As an interferon-inducing, T helper 1 cell-promoting, heterodimeric cytokine, IL-12 connects innate resistance with adaptive immunity by promoting the production of interferon. IL-12 is produced by infected dendritic cells and phagocytes in response to infections as in Figure 2.3 (Trinchieri, 2003). *T. gondii* is a warm-blooded parasite. It

infects vertebrate intermediate hosts, including humans asymptomatically (Tenter *et al.* 2000). In immunocompromised hosts the parasite causes severe toxoplasmosis. Infection in healthy people has an initial phase associated with rapid tachyzoite division capable of infecting practically all nucleated cells. In the long run, persistent infection coincides with an increase in host adaptive immunity (Miller *et al.* 2009). Toxoplasmosis pathogenesis depends on host immune state.

IL-12 has received extensive research, whereas Interleukin-23 and IL-27 have received less attention. A heterodimer of IL-23 and IL-12 is formed by the p40 subunit, but the second subunits p19 and p35 are distinct (Langrish *et al.* 2004). They also share IL-12R1 in their heterodimeric receptors. Macrophages are induced to produce proinflammatory cytokines like TNF and IL-1 by *T. gondii* (Quan *et al.* 2013). Acute and chronic toxoplasmosis resistance necessitates IL-12 for its role in increasing IFN production. In response to *T. gondii* antigens, dendritic cells, neutrophils, and macrophages release IL-12. Toxoplasmosis resistance is primarily mediated by IL-23, a relatively new cytokine with structural similarities to IL-12 however the absence of Interleukin-23 results in only minimal resistance. In innate immunity IL-23 and IL-12 work together (Quan *et al.* 2013).

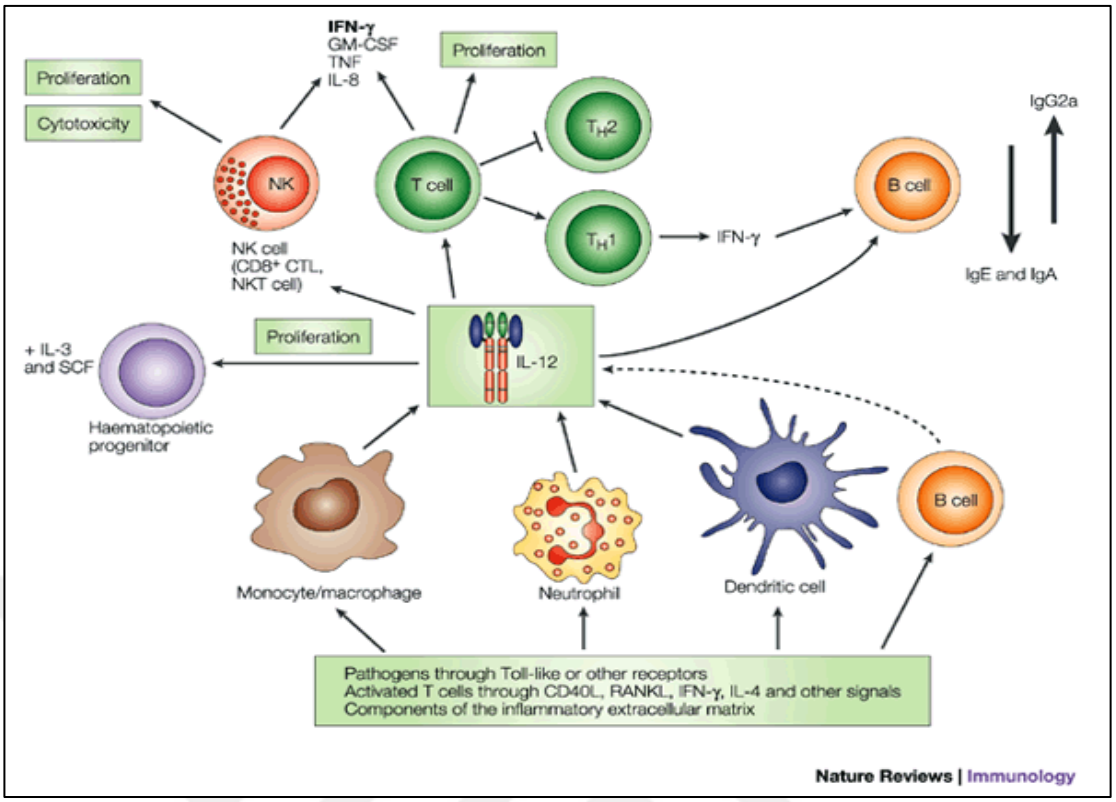

Figure 2.3 Innate resistance and adaptive immunity with IL-12 (Trinchieri, 2003)

DCs, monocytes, and macrophages can all generate IL-23 and IL-12 (Langrish *et al.* 2004). In order to have an effective immune response, it is crucial that these two regulatory cytokines are released at the right time. Cellular and molecular interactions between *T. gondii* and its antigens can control different signaling pathways, resulting in a variety of cell-mediated immune responses (Langrish *et al.* 2004, Quan *et al.* 2013). T. gondii infection has been linked to Interleukin-23 and Interleukin-12 in a few studies, but the production of Interleukin-23 by immune cells infected with T. gondii is unknown. *T. gondii* soluble tachyzoite antigen or excretory/secretory proteins, both at a MOI of 1 parasite-equivalent, were stimulated in THP-1 human monocytic cells, and Interleukin-23 and Interleukin-12 levels were measured by ELISA in response (Quan *et al.* 2013).

2.5.4 *Toxoplasma gondii* IgG and IgM antibodies

T. gondii IgG and IgM antibodies play a crucial role in the immune response against *Toxoplasma* infection (Machumi *et al.* 2017, Vargas-Villavicencio *et al.* 2022). Here is an explanation of the mechanisms behind these antibodies:

T. gondii IgM Antibodies:

Early Immune Response: IgM antibodies are the first antibodies produced during the initial stages of infection or exposure to *T. gondii*. They are primarily generated by naïve B cells (Machumi *et al.* 2017, Vargas-Villavicencio *et al.* 2022).

Specificity: *T. gondii* IgM antibodies are also specific to the antigens present on the parasite's surface. They bind to these antigens, forming antigen-antibody complexes (Machumi *et al.* 2017, Vargas-Villavicencio *et al.* 2022).

Acute Infection Marker: IgM antibodies serve as an important marker for acute or recent *Toxoplasma* infection. Their presence in the bloodstream indicates an ongoing or recent immune response against the parasite (Machumi *et al.* 2017, Vargas-Villavicencio *et al.* 2022).

Limited Duration: IgM antibodies have a shorter half-life compared to IgG antibodies. Their levels typically decrease over time, eventually becoming undetectable after several weeks or months however in some cases IgM antibodies may persist for an extended period (Machumi *et al.* 2017, Vargas-Villavicencio *et al.* 2022).

Indication of Past Infection: In some instances, the presence of IgM antibodies alone may not necessarily indicate an ongoing infection but can be indicative of a previous *Toxoplasma* infection. Further diagnostic tests such as IgG antibody testing or molecular methods are necessary to confirm the current status of infection (Machumi *et al.* 2017, Vargas-Villavicencio *et al.* 2022).

Toxoplasma gondii IgG Antibodies:

Formation: IgG antibodies are produced by B lymphocytes in response to exposure to *T. gondii* antigens. When the parasite enters the body, it stimulates the immune system, leading to the activation and differentiation of B cells into plasma cells that secrete IgG antibodies (Machumi *et al.* 2017, Vargas-Villavicencio *et al.* 2022).

Specificity: *T. gondii* IgG antibodies are specific to the antigens present on the surface of the parasite. These antibodies recognize and bind to the antigens, forming an antigen-antibody complex (Machumi *et al.* 2017, Vargas-Villavicencio *et al.* 2022).

Long-term Immunity: IgG antibodies have a longer half-life compared to other antibody classes, allowing them to persist in the bloodstream for an extended period. They provide long-term immunity against *T. gondii* by neutralizing the parasite, promoting its clearance, and preventing reinfection (Machumi *et al.* 2017, Vargas-Villavicencio *et al.* 2022).

Secondary Response: In case of a subsequent exposure to *T. gondii*, memory B cells specific to the parasite are rapidly activated. These memory B cells differentiate into plasma cells, producing a more robust and rapid IgG antibody response. This secondary response helps in providing enhanced protection against reinfection (Machumi *et al.* 2017, Vargas-Villavicencio *et al.* 2022).

It is worth noting that the detection of IgG and IgM antibodies through serological tests is commonly used for the diagnosis of *Toxoplasma* infection. The interpretation of these antibody results, including the detection of IgM antibodies alone or in combination with IgG antibodies, can help healthcare professionals determine the stage of infection and guide appropriate clinical management and treatment decisions (Machumi *et al.* 2017, Vargas-Villavicencio *et al.* 2022).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Instruments and equipment

All of the devices and equipment used in this study are listed in (Table 3-1).

Table 3.1 The apparatus and equipment employed during present investigation

NO	Device and equipment	Company	Origin
1	ELISA	BioTek	United States
2	plate washer	BioTek	United States
3	Centrifuge	Hettich	Germany
4	Incubator	Thermo Fisher	USA
5	Refrigerator	Hitachi	Japan
6	Shaking Water Bath	Stuart	UK
7	Plain Tube	AFCO	Jordan
8	Gel Tube	AFCO	Jordan
9	Eppendorf Tube 5ml	AFCO	Jordan
10	Multichannel micropipette reservoir	Mybiosource	USA
11	micropipette	Accumax	Europe Union
12	Disposable syringe	TERUMO	Turkey

3.1.2 Kits

All of the research kits used in this study show the (Table no 3-2).

Table 3.2 These are the kits that were utilized for the research

Kit name	Manufacturer	Country of Origin
Human IL-12	Mybiosource	Canada
Anti-Toxoplasma IgG	Mybiosource	Canada
Anti-Toxoplasma IgM	Mybiosource	Canada

3.2 Methods

3.2.1 Study participants

In this case-control study which was conducted between September 2022 and March 2022 at Al-Imameen Al-Kazimin Teaching Hospital, there were a total of 180 volunteers involved. About 90 of these people were healthy and made up the standard study group. The other 90 people had been clinically diagnosed with toxoplasmosis (Figure 3.1). In order to participate in the research, participants gave their verbal permission. Direct interviews are conducted with each patient in order to collect the necessary information.

- Inclusion criteria: Toxoplasmosis patients aged 20-50 years.
- Exclusion criteria: Toxoplasmosis patients aged > 50 and < 20 years with other inflammatory diseases.

3.2.2 Samples

After having four milliliters of blood collected from each participant, the blood was then placed into the gel tube that contained the clot activator, where it was left to coagulate for about ten minutes at room temperature before being centrifuged. Additionally, the serum was separated into five Eppendorf tubes and kept at a temperature of -20 ºC until the quantities of human IL-12, anti-toxoplasmosis antibody (IgG), as well as anti-toxoplasmosis antibody (IgM) were determined.

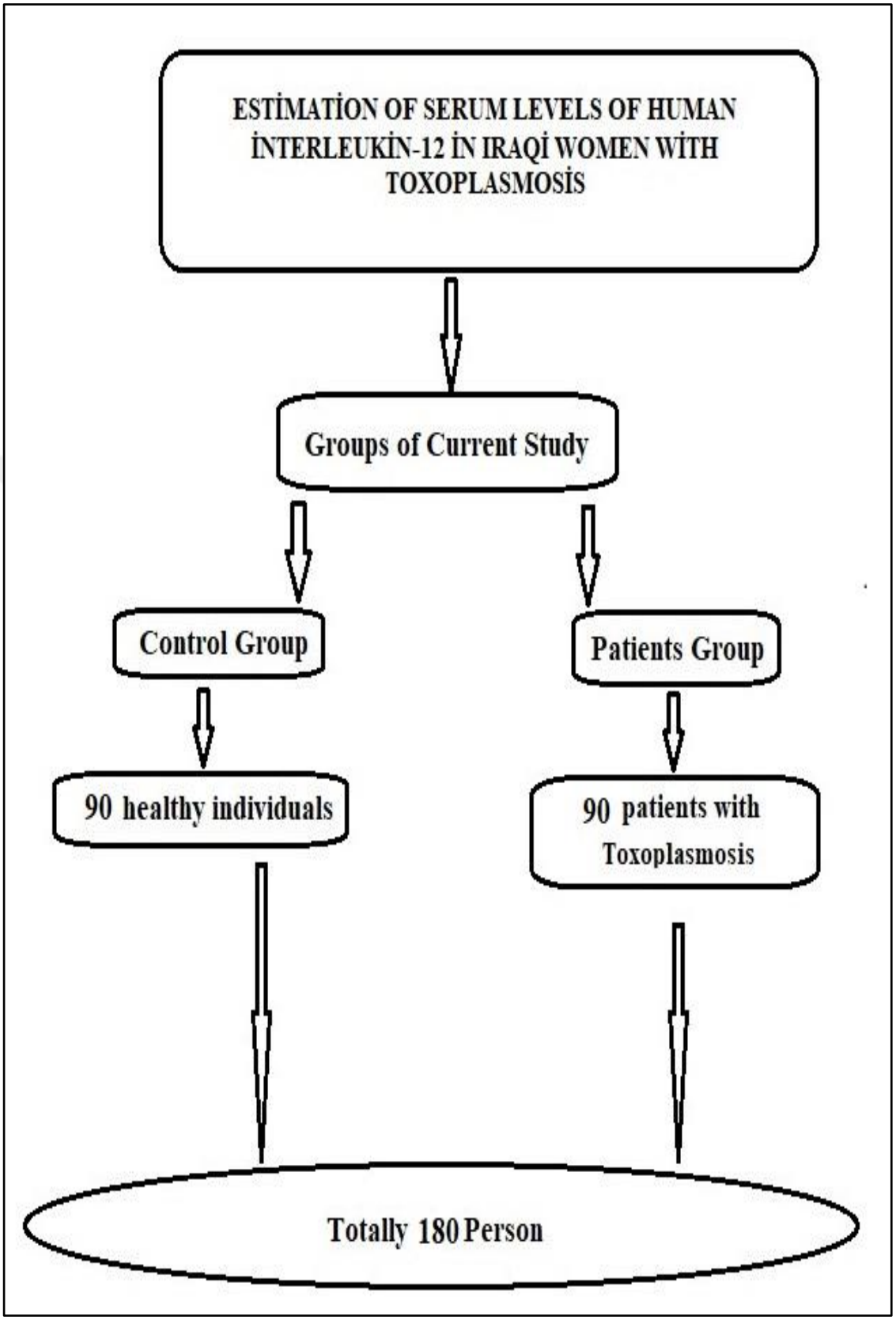

Figure 3.1 The current study structure

3.2.3 Identification of human IL-12 serum concentration

Principle

The sandwich enzyme-linked immunosorbent assay technique was used in the development of this kit. Onto plates containing 96 wells, the capture antibody was pre-coated in advance. In addition, the antibody that was biotin attached was utilized as the detecting antibody. After that, the wash buffer was used to clean the wells after which the standards, test samples and biotin-conjugated detection antibody have been added to the wells. After adding HRP-Streptavidin, unbound conjugates were removed using a wash buffer after the addition of HRP-Streptavidin. TMB substrates were utilized so that the enzymatic response of HRP could be seen. After applying an acidic stop solution, the blue color product that was produced as a result of TMB being catalyzed by HRP turned into a yellow color product. The quantity of material that has to be caught in the plate is relative to the quantity of yellow that is present. After reading the optical density absorbance at 450 nm with a microplate reader, one may figure out how much of the target substance is there.

Procedure

- Before adding wells for the standard, sample, as well as control, the plate was washed twice.
- Transfer 100 µl of the zero tube's contents to the sixth tube, and then transfer blank sample dilution buffer to the standard wells.
- The test sample wells each received 100 µl of a sample that had been appropriately diluted.
- After covering the plate with a lid, the specimen was then heated to 37 ºC for an hour.
- After removing the lid and throwing away the contents of the plate it washed twice with the wash buffer.
- After covering the plate, we incubated it at 37 degrees ºC for an hour with the biotin-labeled antibody working solution that had been added into the wells above.

- After removing the lid, the plate cleaned with wash buffer three times and each time let it sit in the wells for 1-2 minutes.
- Following the addition of 100 µl of SABC Working Solution to each well, the plate was covered and incubated at 37 ºC for thirty minutes.
- After removing the lid and washing the plate five times it was ensured that the wash buffer remained in each well for one to two minutes on each occasion.
- Uploaded 90 µl TMB
- After adding substrate to each well the plate was covered as well as the incubation process was carried out at 37 ºC in the dark around 10–20 minutes.
- Included a volume of 50 ul of Stop Solution in each well. The hue changed to a bright yellow right away.
- When using a microplate reader, immediately after adding the stop solution, read the optical density absorbance at 450 nm.

3.2.4 Identification of anti-*Toxoplasma* antibody (IgG) serum concentration

Principle

After the wells have been coated with pure *Toxoplasma* antigen, the diluted serum is then applied to the wells. In the event that it is present, the toxoplasma IgG specific antibody will attach to the antigen. After the removal of any unbound substances by washing, the enzyme conjugate is next introduced in an effort to attach to any existing antibody-antigen complexes. After washing away any excess enzyme conjugate, the substrate is brought into the reaction. The plate is then allowed to remain in the incubator so that the enzyme may hydrolyze the substrate. The quantity of IgG-specific antibody present in the sample is directly related to the quantity of color that is produced by the reaction.

Procedure

- In order to create a 1:21 dilution of the test samples, add ten microliters of the sample to two hundred microliters of the sample diluent and combined thoroughly.

- In each of the relevant wells, one hundred microliters of diluted sera, calibrator, and controls were dispensed. Dispense one hundred microliters of sample diluent into the first well location (1A) for the reagent blank. Tap the holder to eliminate any air bubbles in the liquid and ensure a thorough mixing. Twenty minutes were spent incubating the sample at room temperature.
- liquid was drained from each of the wells. The wells should each get 300 µL of 1X wash buffer and should be washed three times. Immediately blot the liquid onto absorbent paper and perhaps a paper towel.
- After dispensing 100 µL of enzyme conjugate into each well, the plates were left to incubate at room temperature for twenty minutes.
- Enzyme conjugate was removed from all of the wells. The wells should be washed three times with a total volume of 300 µL with 1X wash buffer. Immediately blot the liquid onto absorbent paper or even a paper towel.
- After dispensing 100 µl of the TMB substrate, the mixture was allowed to incubate at room temperature for ten minutes. We added one hundred microliters of the stop solution.
- In less than 15 minutes, use an ELISA reader to read OD at 450 nm.

3.2.5 Identification of anti-*Toxoplasma* antibody (IgM) serum concentration

Procedure

Following this step, diluted serum is added to each test tube that have been coated using purified antigen. The serum diluent includes sorbent to eliminate Rheumatoid Factor as well as human IgG interference. Antibody that is specific for IgM will attach to the antigen if it is present. After the removal of any unbound substances by washing, the enzyme conjugate is next introduced in an effort to attach to any existing antibody-antigen complexes. The substrate is introduced after the excess enzyme conjugate has been washed away. The plate is then allowed to remain in the incubator so that the enzyme may hydrolyze the substrate. The quantity of IgM specific antibody present in the sample is directly related to the quantity of color that is produced by the reaction.

Procedure

- The calibrator as well as negative positive control are now ready for use. When testing samples, dilute them 1:21 in sample diluent with 10 µl of sample. Make sure everything is well-combined.
- Each well was filled with 100 µl of diluted sera, calibrator, as well as control samples. In the 1A well position, dispense 100 µl of sample diluent. Remove trapped air from the liquid by squeezing the holder. Incubated at room temperature for about 20 minutes.
- All of the wells had liquid removed from them. Using 300 µl of 1X wash buffer, washed the wells three times. Using absorbent paper and otherwise paper towels, swab the surface.
- Each well was filled with 100 µl of enzyme conjugate and incubated at room temperature for 20 minutes.
- A total of every well was flushed with enzyme conjugate. Using 300 µl of 1X wash buffer, washed the wells three times. Using absorbent paper and otherwise paper towels, swab the surface.
- After dispensing 100 µl l of the TMB substrate, the mixture was allowed to incubate at room temp approximately ten minutes.
- administered a stop solution totaling 100 µl.
- Within 15 minutes, use an ELISA reader to read the O.D. at 450 nm.

3.2.6 Statistical analysis

This prospective study's statistical analysis was carried out using the statistical program for the social sciences (SPSS) 21.0 and Microsoft Excel 2013. Mean and standard deviation were the descriptive terms used for the numerical data. The t-test with independent samples was utilized in order to compare the two groups. While the analysis of variance (ANOVA) was employed for research on the differences between the three groups.

The Chi-square test was performed to describe the connection between the variables, and the categorical data were reported as counts and percentages. A Spearman correlation was carried out in order to provide an estimate of the degree of correlation that exists between each pair of variables. 0.05 is the point at which a statistically significant difference is considered to be either nonexistent or unacceptable.

4. RESULTS

4.1 Age of Study Participants

After performing the statistical analysis of the experimental variables, the data of the mean and its standard deviation between those who showed a negative latex test (the healthy ones) and those who showed a positive latex test (infection carriers) were as follows (28.63 ± 7.94 and 28.03 ± 6.74 years) and shown in the table coded by 4.1 and the figure coded by 4.1, where age had no statistical significance among the groups measured in this study, where p-value was 0.585.

Table 4.1 Age of study participants

		Study groups		P value
		Patients	**Controls**	
Age (years)	Mean	28.63	28.03	0.585^{NS}
	Standard Deviation	7.94	6.74	
	Minimum	16.00	16.00	
	Maximum	49.00	44.00	

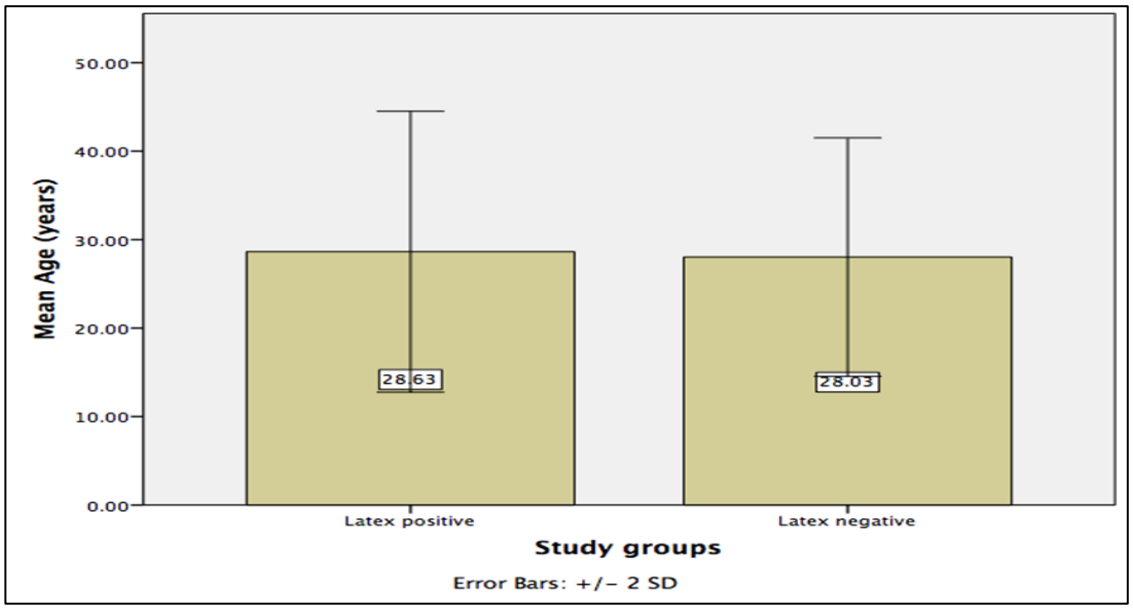

Figure 4.1 Age of study participants

4.2 Age of Study Participants According to Age Groups

After performing the statistical analysis of the experimental variables, the data of the their number with percentage between those who showed a negative latex test (the healthy ones) and those who showed a positive latex test (infection carriers) according to age groups were as follows, the age group of less than 20 years (n = 10, where represents 11.1% for patients, and n = 7, where represents 7.8% for healthy people), the age group 21 to 30 years (n = 47, where represents 52.2% for patients, and n = 54, where represents 60.0% for healthy people), the age group 31 to 40 years (n = 25, where represents 27.8% for patients, and n = 26, where represents 28.9% for healthy people), the age group more than 40 years (n = 8, where represents 8.9 % for patients, and n = 3, where represents 3.3% for healthy people), the table coded 4.2 shows the final results , where age had no statistical significance among the participants according to age groups measured in this study, where p-value was 0.347.

Table 4.2 Age of study participants according to age groups

		Study groups		P value
		Patients	Controls	
Age groups	<=20 years	10	7	0.347^{NS}
		11.1%	7.8%	
	21-30 years	47	54	
		52.2%	60.0%	
	31-40 years	25	26	
		27.8%	28.9%	
	>40 years	8	3	
		8.9%	3.3%	

4.3 IL 12 Concentration Among the Study Participants

After performing the statistical analysis of the IL-12 concentration, the data for the mean and its standard deviation among individuals who showed a negative latex test (healthy individuals) and those who showed a positive latex test (infection carriers) were as follows: 719.08 ± 20.02 and 12.41 ± 0.84 pg/ml, respectively. These results are presented

in Table 4.3 and Figure 4.2. The IL-12 concentration showed a high statistical significance among the measured groups in this study, with a p-value of <0.001.

Table 4.3 Interleukin 12 concentration among the study participants

	Study groups	Mean	Std. Deviation	P value
Interleukin 12 (pg/ml)	Patients	719.08	20.02	<0.001**
	Controls	12.41	0.84	

**: High statistical significance (p<0.001)

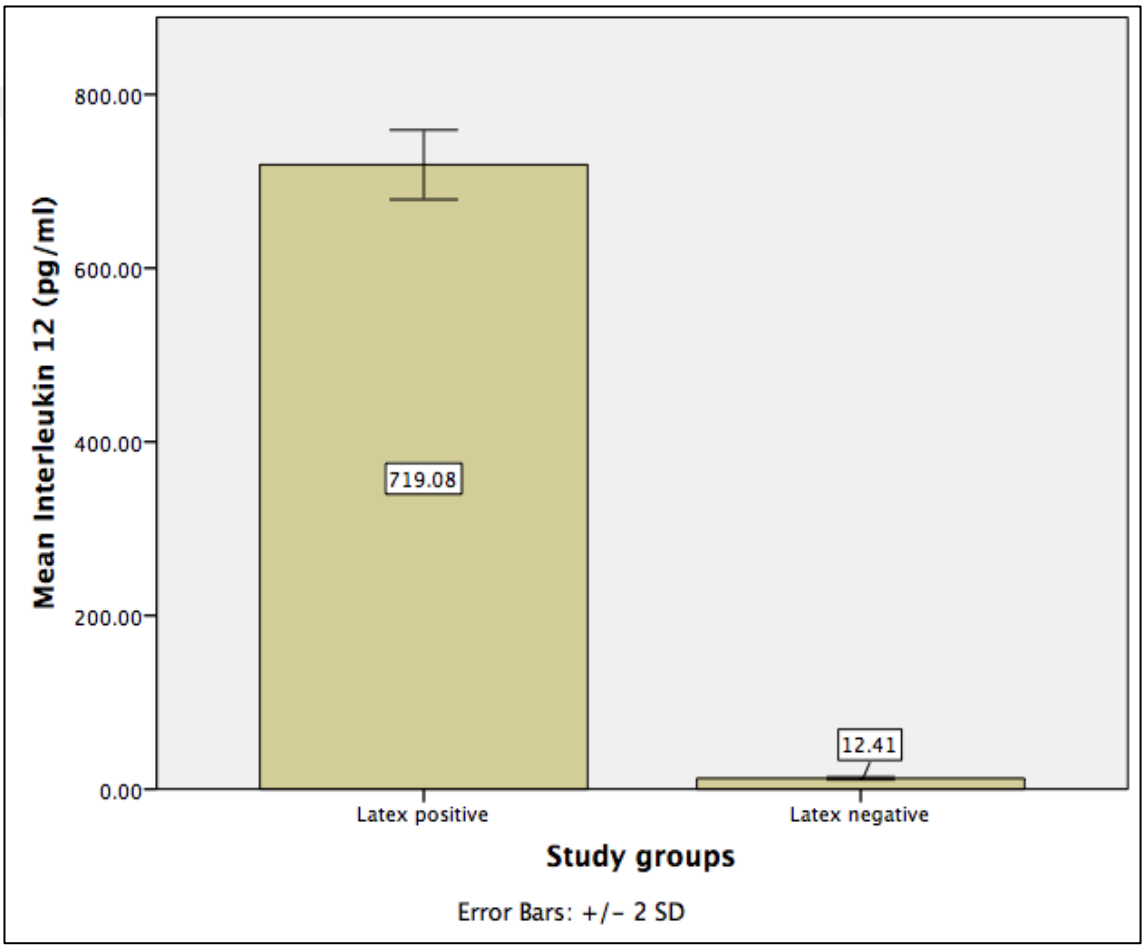

Figure 4.2 Interleukin 12 concentration among the study participants

4.4 Toxoplasmosis IgM Concentration Among Study Participants

After performing the statistical analysis of toxoplasmosis IgM concentration, the data for the mean and its standard deviation among individuals who showed a negative latex test

(healthy individuals) and those who showed a positive latex test (infection carriers) were as follows: 0.39 ± 0.01 and 0.16 ± 0.01 IU/ml, respectively. These results are presented in Table 4.4 and Figure 4.3. The toxoplasmosis IgM concentration showed a high statistical significance among the measured groups in this study, with a p-value of <0.001.

Table 4.4 Toxoplasmosis IgM concentration among study participants

	Study groups	Mean	Std. Deviation	P value
Toxo IgM (IU/ml)	Patients	0.39	0.01	<0.001
	Controls	0.16	0.01	

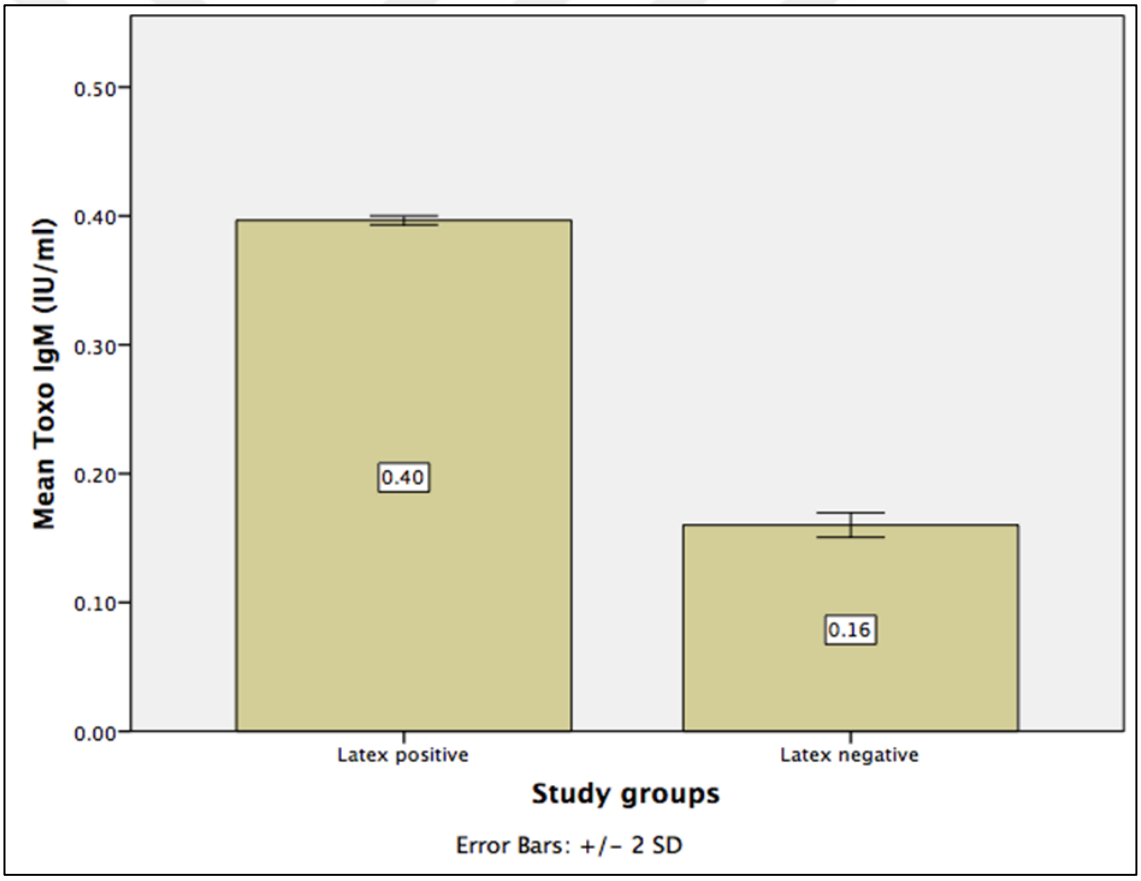

Figure 4.3 Toxoplasmosis IgM concentration among study participants

4.5 Toxoplasmosis IgG Concentration Among Study Participants

After performing the statistical analysis of the toxoplasmosis IgG concentration, the data of the mean and its standard deviation between those who showed a negative latex test (the healthy ones) and those who showed a positive latex test (infection carriers) were as follows (721.48 ± 23.35 and 16.01 ± 0.66 IU/ml) and shown in the table coded by 4.4 and the figure coded by 4.3, where the toxoplasmosis IgG concentration had a high statistical significance among the groups measured in this study, where p-value was <0.001.

Table 4.5 Toxoplasmosis IgG concentration among study participants

	Study groups	Mean	Std. Deviation	P value
Toxo IgG (IU/ml)	Patients	721.48	23.35	<0.001**
	Controls	16.01	0.66	

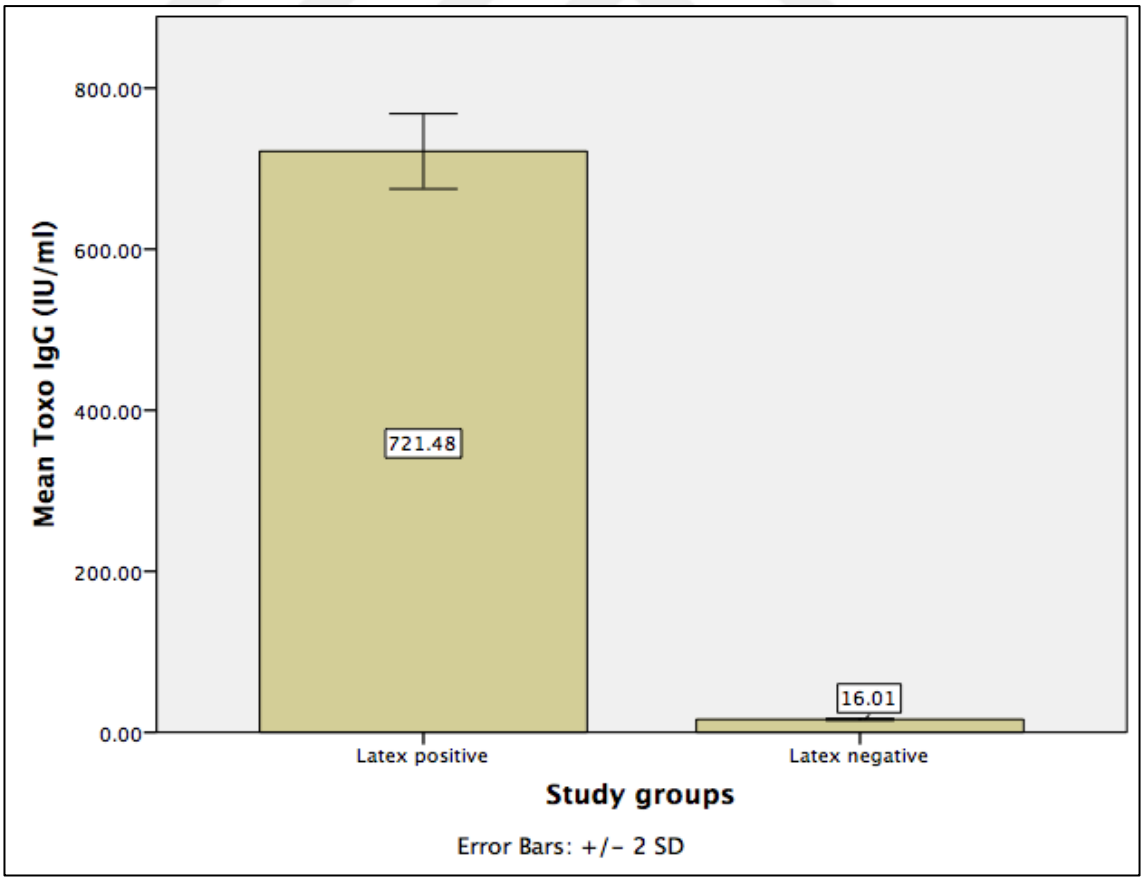

Figure 4.4 Toxoplasmosis IgG concentration among study participants

4.6 Interleukin 12 Concentration Among Study Participants According to Age Groups

After performing the statistical analysis of the interleukin 12 concentration according to the age groups between those who showed a negative latex test (the healthy ones) and those who showed a positive latex test (infection carriers) age groups were as follows, the age group of fewer than 20 years, where IL-12 concentration was (a positive latex= 724.00± 18.15 pg/ml and a negative latex= 12.43± 1.15 pg/ml), and p-value was <0.001, the age group 21 to 30 years (a positive latex= 718.49± 17.02 pg/ml and a negative latex= 12.44± 0.91 pg/ml), and p-value was <0.001, the age group 31 to 40 years (a positive latex= 721.20± 26.26 pg/ml and a negative latex= 12.35± 0.64 pg/ml), and p-value was <0.001, the age group more than 40 years (a positive latex= 709.75± 15.94 pg/ml and a negative latex= 12.53± 0.78 pg/ml), and p-value was <0.001, the figure coded 4.5 and table coded 4.6 shows the final results, where IL-12 concentration had no statistical significance among the participants according to age groups measured in this study, where p-value was 0.453 for positive latex group and IL-12 concentration had no statistical significance among the participants according to age groups measured in this study, where p-value was 0.970 for negative latex group.

Table 4.6 IL-12 concentration among study participants according to age groups

Interleukin 12 (pg/ml)			**Study groups**		**P value**
			Latex positive	**Latex negative**	
Age groups	<=20 years	Mean	724.00	12.43	<0.001**
		SD	18.15	1.15	
	21-30 years	Mean	718.49	12.44	<0.001**
		SD	17.02	0.91	
	31-40 years	Mean	721.20	12.35	<0.001**
		SD	26.26	0.64	
	>40 years	Mean	709.75	12.53	<0.001**
		SD	15.94	0.78	
P value			0.453^{NS}	0.970^{NS}	

NS: none statistical significance (p>0.05)

****:** High statistical significance (p<0.001)

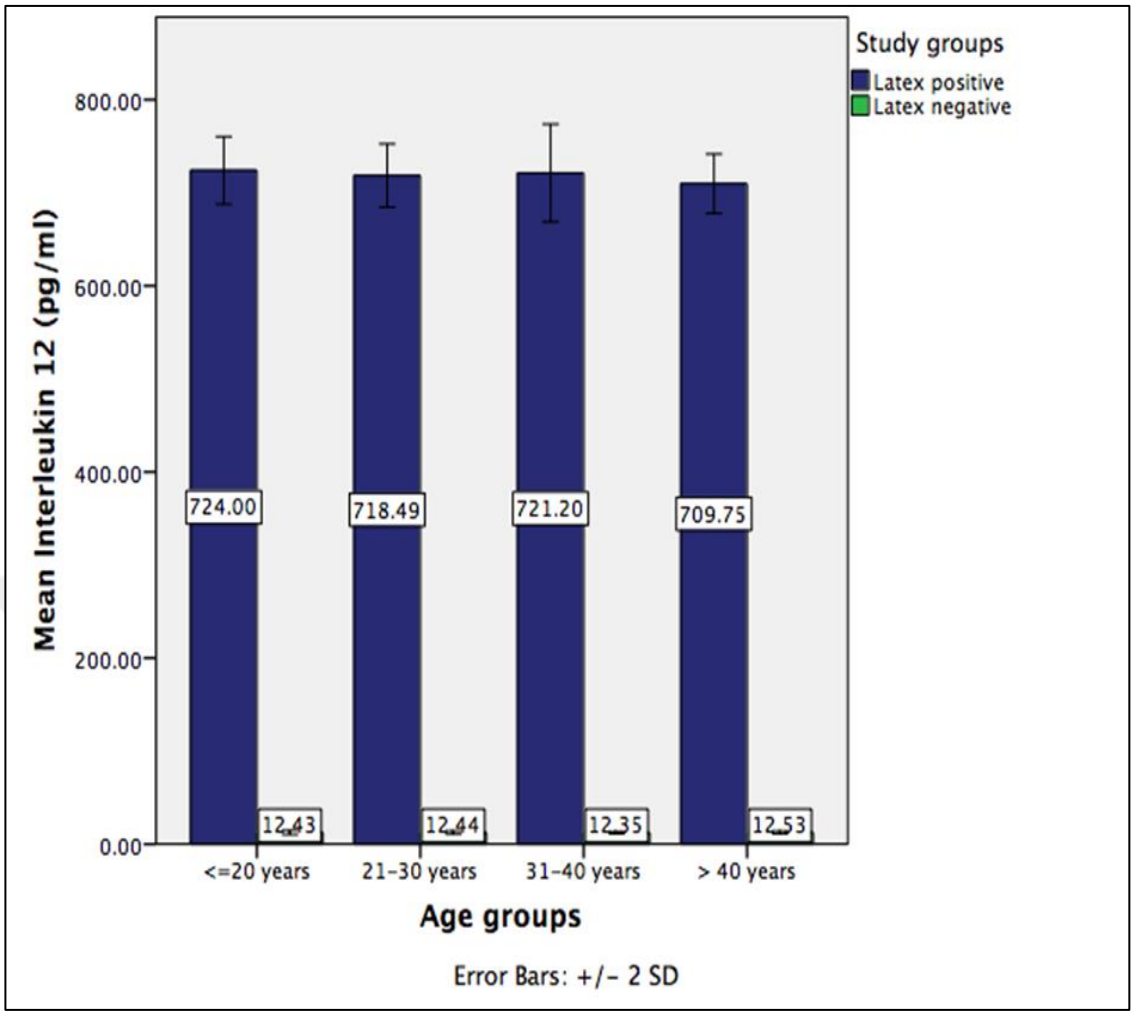

Figure 4.5 IL-12 concentration among study participants according to age groups

4.7 Toxo IgM Concentration Among Study Participants According to Age Groups

After performing the statistical analysis of the toxoplasmosis IgM concentration according to the age groups between those who showed a negative latex test (the healthy ones) and those who showed a positive latex test (infection carriers) age groups were as follows, the age group of fewer than 20 years, where toxoplasmosis IgM concentration was (a positive latex= 0.40± 0.00 pg/ml and a negative latex= 0.16± 0.01 pg/ml), and p-value was <0.001, the age group 21 to 30 years (a positive latex= 0.40± 0.00 pg/ml and a negative latex= 0.16± 0.01 pg/ml), and p-value was <0.001, the age group 31 to 40 years (a positive latex= 0.40± 0.00 pg/ml and a negative latex= 0.16± 0.01 pg/ml), and p-value

was <0.001, the age group more than 40 years (a positive latex= 0.40± 0.00 pg/ml and a negative latex= 0.16± 0.01 pg/ml), and p-value was <0.001, the figure coded 4.6 and table coded 4.7 shows the final results, where toxoplasmosis IgM concentration had no statistical significance among the participants according to age groups measured in this study. Where p-value was 0.738 for positive latex group and toxoplasmosis IgM concentration had no statistical significance among the participants according to age groups measured in this study, where p-value was 0.887 for negative latex group.

Table 4.7 Toxoplasmosis IgM concentration among study participants

Toxo IgM (IU/ml)			Study groups		P value
			Latex positive	Latex negative	
Age groups	<=20 years	Mean	0.40	0.16	<0.001**
		SD	0.00	0.01	
	21-30 years	Mean	0.40	0.16	<0.001**
		SD	0.00	0.00	
	31-40 years	Mean	0.40	0.16	<0.001**
		SD	0.00	0.01	
	>40 years	Mean	0.40	0.16	<0.001**
		SD	0.00	0.01	
P value			0.783 NS	0.887 NS	

NS: none statistical significance (p>0.05)

****:** High statistical significance (p<0.001)

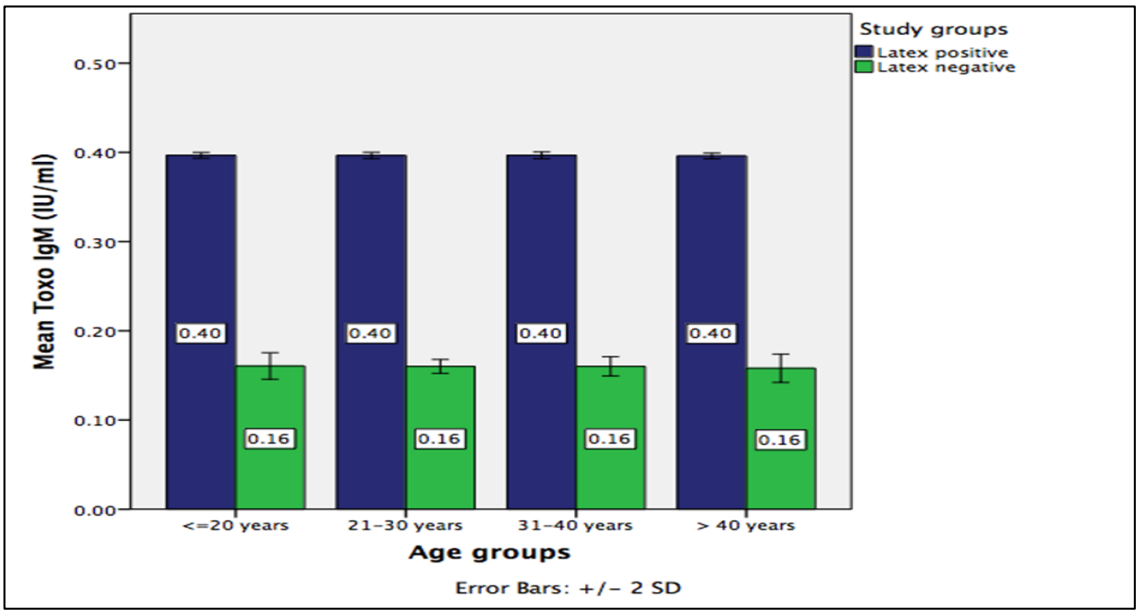

Figure 4.6 Toxoplasmosis IgM concentration among study participants

4.8 Toxo IgG Concentration Among Study Participants According to Age Groups

After performing the statistical analysis of the toxoplasmosis IgG concentration according to the age groups between those who showed a negative latex test (the healthy ones) and those who showed a positive latex test (infection carriers) age groups were as follows, the age group of fewer than 20 years, where toxoplasmosis IgG concentration was (a positive latex= 708.30± 19.57 pg/ml and a negative latex= 15.91± 0.86 pg/ml), and p-value was <0.001, the age group 21 to 30 years (a positive latex= 723.26± 21.22 pg/ml and a negative latex= 15.99± 0.63 pg/ml), and p-value was <0.001, the age group 31 to 40 years (a positive latex= 723.00± 26.21 pg/ml and a negative latex= 16.05± 0.65 pg/ml), and p-value was <0.001, the age group more than 40 years (a positive latex= 722.75± 29.11 pg/ml and a negative latex= 16.30± 1.13 pg/ml), and p-value was <0.001, the figure coded 4.7 and table coded 4.8 shows the final results, where IgG concentration had no statistical significance among the participants according to age groups measured in this study, where p-value was 0.313 for positive latex group and toxoplasmosis IgG concentration had no statistical significance among the participants according to age groups measured in this study, where p-value was 0.831 for negative latex group.

Table 4.8 Toxoplasmosis IgG concentration among study participants

Toxo IgG (IU/ml)			Study groups		P value
			Latex positive	Latex negative	
Age groups	<=20 years	Mean	708.30	15.91	<0.001**
		SD	19.57	0.86	
	21-30 years	Mean	723.26	15.99	<0.001**
		SD	21.22	0.63	
	31-40 years	Mean	723.00	16.05	<0.001**
		SD	26.21	0.65	
	>40 years	Mean	722.75	16.30	<0.001**
		SD	29.11	1.13	
P value			0.313 NS	0.831 NS	

NS: none statistical significance (p>0.05)

****:** High statistical significance (p<0.001)

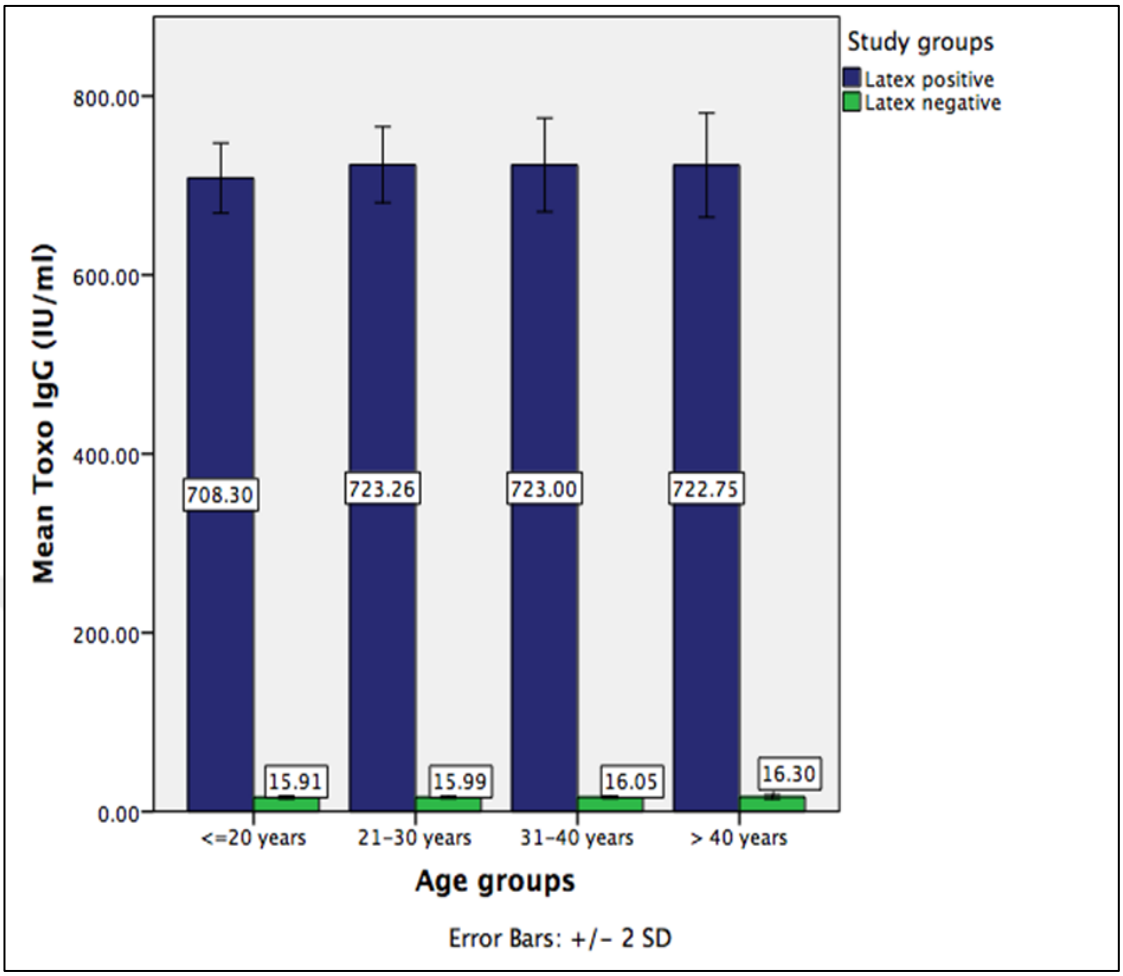

Figure 4.7 Toxoplasmosis IgG concentration among study participants

5. DISCUSSION

5.1 Age of Study Participants

As in clause 4.1, the statistical analysis of the experimental variables, the data of the mean and its standard deviation between those who showed a negative latex test (the healthy ones) and those who showed a positive latex test (infection carriers) were as follows (28.63 ± 7.94 and 28.03 ± 6.74 years) and shown in the table coded by 4.1 and the figure coded by 4.1, where age had no statistical significance among the groups measured in this study, where p-value was 0.585. In a previous Chinese study, which included approximately 125 females suspected of having toxoplasmosis, and their ages ranged from less than 30 years to 45 years, the relationship between the disease and age was not addressed (Zhang *et al.* 2015). In a previous Ethiopian study, it included approximately 255 females infected with toxoplasmosis, where the average age and standard deviation were (31.0 ± 6.5) years, where the 0.05 limit was the standard value as an indication of statistical significance (Zeleke and Melsew, 2017).

5.2 Age of Study Participants According to Age Groups

As in clause 4.2, the statistical analysis of the experimental variables, the data of the their number with percentage between those who showed a negative latex test (the healthy ones) and those who showed a positive latex test (infection carriers) according to age groups were as follows, the age group of less than 20 years (n = 10, where represents 11.1% for patients, and n = 7, where represents 7.8% for healthy people), the age group 21 to 30 years (n = 47, where represents 52.2% for patients, and n = 54, where represents 60.0% for healthy people), the age group 31 to 40 years (n = 25, where represents 27.8% for patients, and n = 26, where represents 28.9% for healthy people), the age group more than 40 years (n = 8, where represents 8.9 % for patients, and n = 3, where represents 3.3% for healthy people), the table coded 4.2 shows the final results , where age had no statistical significance among the participants according to age groups measured in this study, where p-value was 0.347. A Chinese research paper touched on the average

lifespan of women infected with toxoplasmosis, where it found that 27.45% of the infected were within the age group between 31-45, and 12.5% of the toxoplasmosis infected women were in the age group less than 30 years (Zhang *et al.* 2015).

As mentioned in section 4.3, the statistical analysis of IL-12 concentration revealed the following data for the mean and standard deviation between individuals who showed a negative latex test (healthy individuals) and those who showed a positive latex test (infection carriers): 719.08 ± 20.02 pg/ml and 12.41 ± 0.84 pg/ml, respectively. These results are presented in Table 4.3 and Figure 4.2, indicating that IL-12 concentration exhibited high statistical significance among the measured groups in this study, with a p-value of <0.001.

The elevated levels of IL-12 may be attributed to the high virulence of the rapidly reproducing phase of T. gondii. This finding is supported by a study conducted in Iraq, which reported IL-12 levels of 755.76 ± 50.93 pg/ml in patients compared to 16.42 ± 1.48 pg/ml in healthy subjects (Khadem, 2012). Additionally, another study investigating breast cancer patients observed IL-12 levels of 11.520 ± 0.730 pg/ml in patients and 121.080 ± 1.260 pg/ml in healthy subjects (Assim and Saheb, 2020). These findings provide further support for the significant association between IL-12 concentration and the respective health conditions under investigation in the study participants.

5.3 Toxoplasmosis IgM Concentration Among Study Participants

As stated in section 4.4, the statistical analysis of toxoplasmosis IgM concentration revealed the following data for the mean and standard deviation between individuals who showed a negative latex test (healthy individuals) and those who showed a positive latex test (infection carriers): 0.39 ± 0.01 IU/ml and 0.16 ± 0.01 IU/ml, respectively. These results are presented in Table 4.4 and Figure 4.3, indicating that the toxoplasmosis IgM concentration exhibited high statistical significance among the measured groups in this study, with a p-value of <0.001.

An Iraqi study conducted by Khadem (2012) reported toxoplasmosis IgM levels of 0.38 ± 0.002 IU/ml in patients and 0.17 ± 0.02 IU/ml in healthy subjects. This finding further supports the significant association between toxoplasmosis IgM concentration and the respective health conditions under investigation.Toxoplasmosis IgG concentration among study participants

As stated in section 4.5, the statistical analysis of toxoplasmosis IgG concentration revealed that the mean and standard deviation between individuals who showed a negative latex test (healthy individuals) and those who showed a positive latex test (infection carriers) were 721.48 ± 23.35 IU/ml and 16.01 ± 0.66 IU/ml, respectively. These results are presented in Table 4.4 and Figure 4.3, indicating that the toxoplasmosis IgG concentration exhibited high statistical significance among the measured groups in this study, with a p-value of <0.001. An Iraqi study conducted by Khadem (2012) reported toxoplasmosis IgG levels of 776.57 ± 93.2 IU/ml in patients and 13.89 ± 1.6 IU/ml in healthy subjects. This finding further supports the significant association between toxoplasmosis IgG concentration and the respective health conditions under investigation. Additionally, an Iranian study by Assim and Saheb (2020) showed toxoplasmosis IgG levels of 266.360 ± 18.520 IU/ml in patients and 127.580 ± 11.490 IU/ml in healthy subjects. These findings provide additional evidence of the variations in toxoplasmosis IgG concentration between patient and healthy populations. Overall, the statistical analysis and the referenced studies provide strong evidence of the high statistical significance and differences in toxoplasmosis IgG concentrations among the groups examined in this study.

5.4 IL-12 Concentration Among Study Participants According to Age Groups

As in clause 4.6, the statistical analysis of the interleukin 12 concentration according to the age groups between those who showed a negative latex test (the healthy ones) and those who showed a positive latex test (infection carriers) age groups were as follows, the age group of fewer than 20 years, where IL-12 concentration was (a positive latex= 724.00± 18.15 pg/ml and a negative latex= 12.43± 1.15 pg/ml), and p-value was <0.001, the age group 21 to 30 years (a positive latex= 718.49± 17.02 pg/ml and a negative latex=

12.44± 0.91 pg/ml), and p-value was <0.001, the age group 31 to 40 years (a positive latex= 721.20± 26.26 pg/ml and a negative latex= 12.35± 0.64 pg/ml), and p-value was <0.001, the age group more than 40 years (a positive latex= 709.75± 15.94 pg/ml and a negative latex= 12.53± 0.78 pg/ml), and p-value was <0.001, the figure coded 4.5 and table coded 4.6 shows the final results, where IL-12 concentration had no statistical significance among the participants according to age groups measured in this study, where p-value was 0.453 for positive latex group and IL-12 concentration had no statistical significance among the participants according to age groups measured in this study, where p-value was 0.970 for negative latex group. It is possible that the high levels of interleukin-12 is due to the high virulence of the rapidly reproducing phase of Toxoplasma gondii, and this is what was found by an Iraqi study in which the levels of interleukin-12 were (755.76±50.93 pg/ml) in patients, while it was (16.42±1.48 pg/ml) in healthy subjects (Khadem, 2012). In another study, the levels of interleukin-12 were (11.520±0.730 pg/ml) in patients due to breast cancer, while it was (121.080±1.260 pg/ml) in healthy subjects (Assim and Saheb, 2020). We note that there are no significant statistical differences between the levels of interleukin-12 between the age groups, and this was statistically clarified, as the age had no statistical significance with the severity of the infection and the levels of interleukin-12.

5.5 Toxo IgM Concentration Among Study Participants According to Age Groups

As in clause 4.7, the statistical analysis of the toxoplasmosis IgM concentration according to the age groups between those who showed a negative latex test (the healthy ones) and those who showed a positive latex test (infection carriers) age groups were as follows, the age group of fewer than 20 years, where toxoplasmosis IgM concentration was (a positive latex= 0.40± 0.00 pg/ml and a negative latex= 0.16± 0.01 pg/ml), and p-value was <0.001, the age group 21 to 30 years (a positive latex= 0.40± 0.00 pg/ml and a negative latex= 0.16± 0.01 pg/ml), and p-value was <0.001, the age group 31 to 40 years (a positive latex= 0.40± 0.00 pg/ml and a negative latex= 0.16± 0.01 pg/ml), and p-value was <0.001, the age group more than 40 years (a positive latex= 0.40± 0.00 pg/ml and a negative latex= 0.16± 0.01 pg/ml), and p-value was <0.001. The figure coded 4.6 and table coded

4.7 shows the final results, where toxoplasmosis IgM concentration had no statistical significance among the participants according to age groups measured in this study. Where p-value was 0.738 for positive latex group and toxoplasmosis IgM concentration had no statistical significance among the participants according to age groups measured in this study where p-value was 0.887 for negative latex group. An Iraqi study showed that the levels of toxoplasmosis IgG were (776.57±93.2 IU/ml) in patients, while it was (13.89±1.6 IU/ml) in healthy subjects (Khadem, 2012). An Iranian study showed that the levels of toxoplasmosis IgG were (266.360±18.520 IU/ml) in patients, while it was (127.580±11.490 IU/ml) in healthy subjects (Assim and Saheb, 2020). We note that there are no significant statistical differences between the levels of toxoplasmosis IgM concentration between the age groups, and this was statistically clarified, as the age had no statistical significance with the severity of the infection and the levels of toxoplasmosis IgM concentration.

5.6 Toxo IgG Concentration Among Study Participants According to Age Groups

As described in section 4.8, the statistical analysis of toxoplasmosis IgG concentration according to age groups revealed the following data for individuals who showed a negative latex test (healthy individuals) and those who showed a positive latex test (infection carriers): (a positive latex= 708.30± 19.57 pg/ml and a negative latex= 15.91± 0.86 pg/ml), and p-value was <0.001, the age group 21 to 30 years (a positive latex= 723.26± 21.22 pg/ml and a negative latex= 15.99± 0.63 pg/ml), and p-value was <0.001, the age group 31 to 40 years (a positive latex= 723.00± 26.21 pg/ml and a negative latex= 16.05± 0.65 pg/ml), and p-value was <0.001, the age group more than 40 years (a positive latex= 722.75± 29.11 pg/ml and a negative latex= 16.30± 1.13 pg/ml), and p-value was <0.001. The figure coded 4.7 and table coded 4.8 shows the final results, where IgG concentration had no statistical significance among the participants according to age groups measured in this study, where p-value was 0.313 for positive latex group and toxoplasmosis IgG concentration had no statistical significance among the participants according to age groups measured in this study, where p-value was 0.831 for negative latex group. An Iraqi study showed that the levels of toxoplasmosis IgG were

(776.57±93.2 IU/ml) in patients, while it was (13.89±1.6 IU/ml) in healthy subjects (Khadem, 2012). An Iranian study showed that the levels of toxoplasmosis IgG were (266.360±18.520 IU/ml) in patients, while it was (127.580±11.490 IU/ml) in healthy subjects (Assim and Saheb, 2020). We note that there are no significant statistical differences between the levels of toxoplasmosis IgG concentration between the age groups, and this was statistically clarified, as the age had no statistical significance with the severity of the infection and the levels of toxoplasmosis IgG concentration.

6. CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

1. There is no relationship between age and severity of Toxoplasmosis infection.
2. There is no age group more susceptible to infection than another group within the reproductive age of women with Toxoplasmosis.
3. The level of IL-12 in the serum of infected females with toxoplasmosis is directly proportional to the severity of the infection.
4. It is possible to use the quantitative measurement of IL-12 as an indicator to measure the severity of infection in women infected with toxoplasmosis.
5. The latex test gave results identical to the immunoglobulin assays.
6. The level of toxoplasmosis IgM in the serum of infected females with toxoplasmosis is directly proportional to the severity of the infection.
7. The level of toxoplasmosis IgG in the serum of infected females with toxoplasmosis is directly proportional to the severity of the infection.

6.2 Recommendations

1. We recommend conducting more tests and experiments on women infected with toxoplasmosis and examining more interleukins that may be elevated during the parasitic infection.
2. It is possible to conduct medical follow-up of patients from diagnosis of infection to complete recovery and evaluation of IL-12 values in serum.

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