بسم الله الرحمن الرحيم

Shendi University

College of Graduate Studies and Scientific Research

Serodiagnosis and cytokine (IL-8 and IL-17) levels in pregnant women with Toxoplasmosis in Khartoum state- Sudan

التشخيص المصلى وقياس مستويات السيتوكينات (**IL-8 and IL-17**) عند النساء الحوامل المرضى بداء القطط فى ولاية الخرطوم- السودان

Athesis submitted in fulfilment for the degree of PhD in Medical Laboratory Sciences- Parasitology and Medical Entomology

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قال تعالى :

(إِنَّ اللَّهَ وَمَلَائِكَتَهُ يُصَلُّونَ عَلَى

النَّبِيِّ يَا أَيُّهَا الَّذِينَ آمَنُوا

صَلُّوا عَلَيْهِ وَسَلِّمُوا تَسْلِيمًا)

صدق الله العظيم

الاحزاب اية (56)

Dedication

To my

** Beloved and blessed Parents who did everything for me*

** Dear brothers and sisters.*

** Lovely Grand mother (Rogia).*

** Dr. Amged Mohamed Abelkarrem*

** Dear Abuobida*

** Dear Yousif Kassab*

**Dear Walaa and ahmed*

**Dear hanoo*

Idedicate this work.

Aknoweldgement

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

I would like to express my thanks and gratitude to my supervisors, Professor. Hamid Suliman Abdalla, for his continuos encouragement and invaluable advices during the various stages of this work.

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Abstract

This study was carried out during the period from May 2015 to April 2017 in Khartoum state. It's a comparative cross-sectional one designed to assess immunoglobulins and cytokines in pregnant women. A total of 300 venous blood samples were collected from each pregnant woman and centrifuged to obtain serum. Each serum sample was investigated using three serological methods: Toxolatex, ICT and ELISA. Patient's information was recorded in a questionnaire previously designed for the purpose of analysis. In addition, 40 uninfected women were enrolled in the study as control group to assess the level of IL8 and IL17 cytokines. The overall seropositivite rate of *Toxoplasma gondii* infection was 22.6%, 30% and 15.3% using ELISA, Toxolatex and ICT respectively. The higher rate of infection was encountered within the age group from 21 to 30 years using ELISA. There was no statistically association between toxoplasmosis and cat contact, consumption of raw meat and history of miscarriage.

Within the positive cases of study population, only 16 and 13 showed positive results of IL8, IL17 respectively. The results showed highly significant increase in the mean serum level of IL8 (210.25pg/ml) and IL17 (203.15 pg/ml) when compared to the control group who showed 68.9 pg/ml and 54.8 pg/ml respectively. The serum level of proinflammatory cytokines investigated in this study seems to be increased in patients with serological evidence of *Toxoplasma gondii* infection.

As a general conclusion, it can be stated that the frequency of specific IgM and IgG antibodies using ELISA is high among pregnant women investigated for toxoplasmosis.

ملخص الدراسة

اجريت هذة الدراسة خلال الفتره من مايو 2105 الي ابريل 2107 في ولايه الخرطوم. وكان الهدف من هذه الدراسه المقطعيه هو التحقق من وجود وانتشار الاجسام المضاده والمتخصصه في مرض داء القطط..كذلك تهدف الدراسه الي التحقق من مستويات بعض السيتوكينات (IL8and IL17).وشملت الدراسه جمع 300عينه من الدم الوريدي من الحوامل وتدويرها للحصول علي المصل.تم الكشف عن الاجسام المضاده في هذه الامصال باستخدام ثلاثه طرق مختلفه هي توكسولاتكس ,ايستي ,اليزا.

وقد تم تسجيل بعض بيانات المرضي في استماره استبيان تم تصحيحها مسبقا لتستخدم في التحليل. بالاضافه الي ذلك تم ادراج 40من الحوامل غير المصابين كمجموعه ضابطه وذلك لتقييم مستويات السيتوكينات. وقد اوضحت النتائج ان معدل الحالات الموجبه وقد كان 22.6%و30%و15.3% باستخدام تقنيات هي توكسولاتكس ,ايستي ,اليزا.

وقد كانت اعلي معدلات الاصابه في المجموعه العمريه من 21-30 سنه باستخدام تقنيه . لم تظهر نتائج الدراسه اي علاقه واضحه بين داء القطط وبعض عوامل الخطرالمسببه للمرض مثل) الملامسه المباشره للقطط وتناول اللحوم غير المطهيه) والتعرض لحالات اجهاض من قبل.وقد خلصت الدراسه الي ان مستويات الاجسام المضاده عند الاصابه بداء القطط تزادد بصوره واضحه عند الحوامل. وجد خلال العينا ت الموجبه من الحوامل المصابين بداء القطط ان هنالك فقط 16 و13 حاله موجبه تحتوي امصالها علي السيتوكينات انترلوكين 8وانتراوكين17 .علي الترتيب. وقد اوضحت النتائج كذلك ان هنالك زياده زات مغزي واضح في مستويات هذه السيتوكينات مثلا متوسط مستوي انترلوكين8 هو 210بيكو جرام ومتوسط مستوي انترلوكين 17 هو 203 بيكوجرام مقارنه مع العينه الضابطه التي اظهرت فقط 68.9 بيكوجرام و 54.8 بيكوجرام علي الترتيب. .كما خلصت الي ان السيتوكينات التي تم الكشف عنها توضح زياده طرديه مقارنه مع الاجسام المضاده للمرض.

Contents

List of figures

List of tables

Chapter one

Introduction

Rationale

Objectives

1.1 Introduction

Toxoplasmosis is a zoonotic disease caused by the protozoan parasite *Toxoplasma gondii*. Humans and other warm blooded animals are intermediate hosts (Steven, *et al*., 2008).The infection has a worldwide distribution. Approximately one-third of all humanity has been exposed to this parasite, but the seroprevalence varies considerably between countries from less than 10% to more than 90%. (Rosso, *et al*., 2008). Toxoplasmosis in human was identified in Sudan when Carter and Fleck in 1966 used the Dye test (DT) and reported the prevalence of 61% in four different states in the country, Abdelhakam *et al*; (2016) examined serum samples collected from pregnant and gynecological women in Tendalty, White Nile State and the overall prevalence of parasite were found to be 38.5%. All mammals, including humans, and birds are intermediate hosts, whereas Feline (cats) are definitive host, being the only animals that pass oocyst in their feces. Sheep and goat meats are important infection sources for toxoplasmosis (Sevgili, *et al*; 2005). *T. gondii* infects humans via ingestion of undercooked meat containing trophozoites or tissue cysts, or through ingestion of sporulated oocysts found in food, soil, and water recently contaminated with cat feces (Robert-Gangneux and Darde*; 2012;* Jones and Dubey; 2010; Lass *et al*; 2009). When oocysts are ingested by an intermediate host such as humans (Hill and Dubey*; 2002*), they migrate in the host's body and form pseudocysts in various organs and tissues, but most commonly in the brain (Webste*r, et al;* 2006*).* Toxoplasmosis is a lifelong infection that has the potential to reactivate and cause severe disease and even death in immunocompromised individuals. Humans may remain infected for life and will stay asymptomatic unless immunosuppression occurs (Herrmann, *et al*; 2010). Primary infection of toxoplasmosis in immunocompetent subject is usually asymptomatic or

associated with self limited symptoms such as fever, malaise, and cervical lymphodenopathy. Infection acquired during pregnancy is frequently associated with transmission of *T.gondii* to the fetus, resulting in congenital disease. In immunocompromised patients, *T. gondii* infection causes severe manifestation, including spleenomegaly, chorioretinitis, pneumonitis, encephalitis, multisystem organs failure, and even death (Montoya and Liesenfeld, 2004). *T. gondii* infection has great importance in public health. Women in initial stages of gestation may undergo miscarriage, premature birth, neonatal death (Jones, *et al*; 2001; Soares, *et al;* 2011).This parasite may also cause behavioral changes (Lafferty, 2005). During pregnancy, cases of antenatal depression and anxiety have been related Groer *et al;*2011 ; Campello, *et al;* 2012 ; Faucher, *et al;* 2012 and Lopes-Mori, *et al;* 2013). In patients infected with human immunodeficiency virus (HIV), more than 90% of *Toxoplasma* encephalitis cases involved reactivation of a latent infection (Luft and Chua, 2000), depending on various factors such as, age, sociocultural and nutritional habits, contact with domestic cats, climatic and geographical conditions (Barbosa, *et al.*, 2009). *T.gondii* is recognized by IgG, IgM, IgA and IgE antibodies in patients with acute and chronic toxoplasmosis depending on the stage of the parasite (Singh, 2003). As the parasite cannot be detected microscopically, diagnosis relies on serological methods such as the latex agglutination (LA) test, ELISA, and IFAT. Detection of toxoplasmosis utilizing IgM antibodies is the most common method used to determine if infection is current. The IgM antibodies rise in response to infection, and may be persistently elevated for many months. A negative IgM result during pregnancy is most reassuring that acute infection has not occurred (Del Bono, *et al*; 1989), it is therefore essential to estimate

the time of infection as precisely as possible to properly manage the risk to the patients.(Flegr, *et al*., 1996).

Chemokines are a group of chemotactic polypeptides that are key mediators of leukocyte activation and chemotaxis (Tenter, *et al;* 2000; Šárka, *et al*; 2007). IL8 has an important role in the innate immune response. Interleukin-8 is often associated with inflammation. It has been cited as a pro-inflammatory mediator in toxoplasmosis (Tenter, *et al;* 2000). It is well recognized that T cell-mediated immunity plays a central role in the host response to intracellular pathogens (Šárka *et al;* 2007). T cell- mediated immunity and activated macrophages have been shown to play important roles in resistance to T cell-mediated immunity *T. gondii* infection (Bliss *et al;* 2001; Ju *et al*; 2009 and Denkers and Gazzinelli*;* 1998). Initial innate immune response led by neutrophils has also been reported to be critical for successful resolution of the infection (Bliss, *et al;* 2001). Several studies have shown that their loss leads to exacerbation of infection (Kelly, *et al;* 2005). The early induction of neutrophil induction during *T. gondii* infection is dependent on IL-17-mediated signaling. The neutrophils clear the parasites during initial stages of infection. (Yisong and Richard, 2009).

It is important to confirmation of the presence of the parasite, especially among pregnant women is essential for treatment to prevent the transmission to the fetus (Jennifer, *et al.,* 2003). It is important to confirmation of the presence of the parasite, especially among pregnant women is essential for treatment to prevent the transmission to the fetus (Jennifer, *et al.,* 2003).

1.2 Rationale

T. gondii readily can infect human beings. Those particularly at risk of developing clinical illness include pregnant women through multiplying of the parasites in the placenta and fetus .The parasite can pose a serious threat to the unborn child that may result in misscarge. This reflect the increase the cases of repeated misscarge cases recently. In individuals who are immunosuppressed, such as tissue transplant patients, AIDS patients and those undergoing certain forms of cancer therapy, toxoplasmic encephalitis is aserious clinical complication.

The infection can be diagnosed using serum, aminotic fluid, fetal membrane, cerebrospinal fluid and saliva. Demonstration of specific IgG and IgM anti-*Toxoplasma* antibodies in serum of suspected patients may be helpful in diagnosis considering how widespread in Sudan. Most of the research on toxoplasmosis was geared to prevlance and epidmology of the disease with special emphasis on its effect in pregnant women. Cytokines play considerable role in toxoplasmosis, whether proinflammatory or regulatory responces in *Toxoplasma* infection, despite that very few studies to examine cytokines and to assess their role in immunity in toxoplasmosis is not explored. This study may constitute a preliminary trial on two cytokines IL8 and IL17 in toxoplasmosis in pregnant women in Sudan.

1.3 Objectives

1.3.1 General objective:

To conduct serodiagnosis on *T.gondii* and estimate cytokines (IL8&IL17) levels in pregnant women in Khartoum state, Sudan.

1.3.2 Specific objectives:

1. To detect the infection rate of *T. gondii* infection among pregnant women.
2. To assess the risk factors associated with toxoplasmosis.
3. To determine the level of IL8 and IL17 in pregnant women.

Chapter two

Literature review

2.1 Historyof *Toxoplasma gondii*:

T. gondii was first discovered by Nicolle and Mancesux 1908 in tissues of a hamster-like rodent, the gundi (*Ctenodactylus gundi*), which was being used for leishmenasis resarch in the laboratoryof Charles Nicolle at the Pasteur institute in Tunis. The name of *T. gondii* was coined by Nicolle and Mancesux 1908 based on the crescent shape of the tachyzoite (in Greek: toxo= arc or bow, plama=form or life). At about the same time, Splendore (1908) working in Sao Paulo, Brazil, discovered a similar parasite in rabbits also erroneously identifying it as *leishmenia*, but he did not name it (Dubey, 2010).The medical importance of *T. gondii* remained unknown until 1939 when it was identified in tissues of a congenitally- infected infant presenting with the classic triad of symptoms, namely hydrocephalus, retinochoroiditis and intracranial calcification (Dubey, 2008 and Innes, 2010b).The discovery *T. gondii* specific antibody test , sabin-feldman dye test, in 1948 led to the recognition that *T. gondii* is acommon parasite of warm –blooded hosts with worldwide distribution (Dubey,2008).The veterinary impotence of *T. gondii* became known when it was found to cause abortion storms in sheep in 1957(Dubey,2008).Evidence for the coccidian nature of *T. gondii came* first from electron microscopic studies carried out in the 1960s, which revealed ultrastructural similarities between extra intestinal merozoite of *T. gondii* and intestinal merozoite of *Eimeria* species, and thus indicated coccidian –like life cycle for *T. gondii (*Tenter, *et al*; 2000). In 1970, knowledge of *T. gondii* life cycle was completed by the discovery of the sexual phase of the parasite in the small intestine of the cat (Dubey, 2008). In the 1980s, *T. gondii* emerged as a major cause of death in patients with acquired immunopathology syndrome, illustrating the importance of the immune

system in controlling *T. gondii* infection (Innes, 2010b). The discovery of the common *T. gondii* in certain marine wildlife indicates contamination of seas with *T. gondii* oocysts washed from land (Dubey, 2008). Now adays the parasite is found in almost every country of the world and in all warm-blooded animals including humans (Dubey and Beattie, 1988 and Innes, 2010 a).

2.2 *Toxoplasma gondii* classification:

T. gondii, is classified according to Ferguson (2002) as follows:

Kingdom	Animalia
Sub Kingdom	Protozoa
Phylum	Apicomplexa
Class	Sporozoea
Subclass	Coccidia
Order	Eucoccidea
Suborder	Eimeriina
Family	Sarcocystidae
Genus	*Toxoplasma*
Species	*gondii*

2.3 Mode of transmission:

The principal modes of *T. gondii* transmission are ingestion of faecal oocysts or tissue cysts, and the transplacental transmission of tachyzoites from mother to unborn child. Infection with faecal oocysts may occur by accidentally ingesting contaminated soil (e.g. not washing hands after gardening or eating

unwashed fresh produce), drinking untreated contaminated water, eating shellfish grown in contaminated water, or contact with cat faeces. Infection from tissue cysts may occur by consuming raw or undercooked meat, by accidentally consuming tissue cysts after handling raw meat and not washing hands thoroughly, or by cross-contamination of food prepared using unwashed utensils and chopping boards that have had contact with raw meat (Abu-Madi, *et al*; 2010 and Pereira, *et al*; 2010). Oocyst-acquired infections in humans are clinically more severe than tissue cyst-acquired infections (Dubey, 2004). As tachyzoites are sensitive to environmental conditions they are usually killed rapidly outside the host and so are rarely involved in food borne transmission of *T. gondii* (Tenter, 2009). Another route of transmission of *T. gondii* infection in humans is via congenital infection and is often associated with severe pathology in the fetus. In humans, transmission of *T. gondii* from the mother to the fetus is considered to be most efficient during the last trimester of pregnancy but clinical congenital toxoplasmosis is more severe if transmission occurs during the first trimester.

Organ transplant recipients can develop toxoplasmosis due to transmission of the parasite with the transplanted organ from a *Toxoplasma*-seropositive donor to a *Toxoplasma*-seronegative recipient. Heart transplantation is the most common type of organ transplantation procedure when this occurs, as cysts form in the cardiac muscles. (Martina, *et al;* 2011 and Derouin and Pelloux, 2012). However, toxoplasmosis is an uncommon outcome from organ transplantation as only 5% of human pathogenic parasites have reportedly caused significant illness in transplant recipients (Barsoum, 2006).It is also possible that parasite transmission could occur as the result of blood transfusion or hematopoietic stem cell transplantation. The chance of

either of these occurring is very low and could only occur if the donor was recently infected with *T. gondii* and so had tachyzoites present in their blood and bone marrow (Derouin and Pelloux, 2012).

2.4 Life cycle of *T.gondii:*

Members of cat family are the only known definitive hosts for the sexual stages of *T. gondii* and thus considered the main reservoirs of infection. Infection of the feline definitive host occurs when a cat consumes an intermediate host (such as a mouse or bird) infected with tissue cysts. Upon ingestion of a tissue cyst by a susceptible cat, the walls of the cyst are digested by proteolytic enzymes and bradyzoites are released. The bradyzoites undergo asexual reproduction followed by sexual reproduction in intestinal epithelial cells to produce micro gametocytes and macro gametocytes. The microgametocytes fertilize the macro gametocytes, leading to the production of zygotes. The zygotes differentiate into unsporulated oocysts and are shed in the faeces of the definitive host (Ortega, 2007; Jones and Dubey, 2010). The unsporulated oocyst takes 1-5 days after excretion to sporulate and become infective (Ruelhmann, 2010).

After a prepatent period of up to 10 days following primary infection with tissue cysts, a cat may shed more than 100 million oocysts into the environment over a 2-3 week period (Tenter, *et a*l; 2000).

Oocyst can survive in the environment for several months and are remarkably resistance to disinfectant, freezing and drying (Dubey *et al.,* 2004). Inside the intermediate host the parasite form tissue cyst, most commonly in skeletal muscle, myocardium and brain, these cysts may remain throughout the life of the host (Schmidt *et al* ,. 2005). There are three infectious stages of *T. gondii*:

the tachyzoites, the bradyzoites and the sporozites. They are linked in a complex life cycle which is illustrated in Figure 2.1

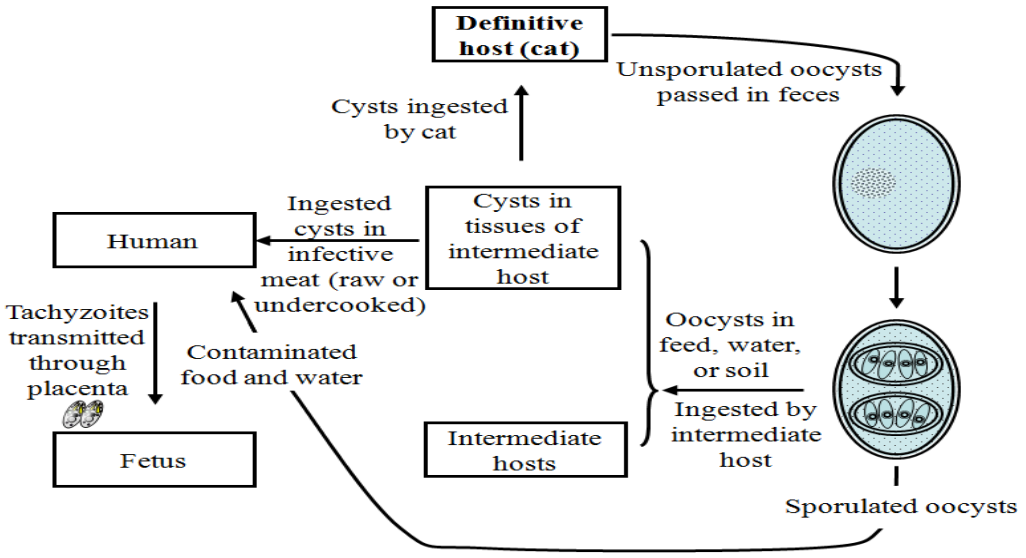

Figure (2.1): Life cycle of *T. gondii* (Adapted from Dubey *et al*; 1985).

2.5 Virulence and infectivity:

T. gondii virulence and infectivity are reliant on factors that control parasite-host cell interactions and/or moderate the host immune response (Dubremetz and Lebrun, 2012).The population structure of *T. gondii* is comprised of three highly abundant and overrepresented genetic lineages, commonly referred to as genotypes I, II and III, amongst a diverse array of related genotypes (Su, *et al;* 2012).The three clonal lineages are very closely related but the small genetic differences result in distinct phenotypic differences in infectivity and virulence (Sibley and Ajioka, 2008).

Most studies on virulence have involved genotypes I, II and III and virulence has typically been assessed in a mouse pathogenicity model, with comparatively little known about human infection (Dubremetz and Lebrun, 2012). In the mouse model, highly virulent strains are typically genotype I

whereas the vast majority of non-virulent strains are genotype II and III (Sibley and Boothroyd, 1992). Little is known about atypical or recombinant genotypes (Dubremetz and Lebrun, 2012). In humans, the evidence for strain specific virulence is less well studied and relies predominantly on epidemiological evidence. The majority of human cases have been attributed to genotype II (Howe and Sibley, 1995), which is likely to be an artefact of an over representation of this genotype in animals in Europe and the United States (US) where most human cases have been documented (Boothroyd and Grigg, 2002). The virulent nature of genotype I strains in mice may, however, extend to humans as severe ocular disease in otherwise immunocompetent adults have been attributed to genotype I strains (Boothroyd and Grigg, 2002).

Furthermore, non-genotype II strains have been associated with more severe disease at birth in congenitally infected newborns in the US (McLeod, *et al*; 2006). More recently, highly virulent atypical genotypes in French Guiana and Brazil have caused severe disease in immunocompromised individuals, foetuses and otherwise healthy individuals (Carme, *et al;* 2009; Dubey, *et al*; 2013). In Australia, genotype II strains have been reported from a human isolate (Sibley and Boothroyd 1992) and a dog isolate (Al-Qassab, *et al;* 2009) and atypical and type II-like strains have been isolated from native Australian wildlife .Of the few Australian isolates examined thus far, all have been avirulent in mouse bioassays(Parameswaran, *et al*; 2010).

2.6 Epidemiology:

T. gondii has a worldwide distribution, but its prevalence varies greatly (Jones, *et al*; 2001). Sero prevalences of 43%and 28.3% have been reported in European countries such as France and Italy, respectively (Berger, *et al*;

2009; De Paschale, *et al*; 2010).In Thailand, report showed 29.1 % prevalence of toxoplasmosis in pregnant women (Nissapatorn, *et al*; 2011) whereas in Brazil Barbosa, *et al;* (2009) reported a prevalence rate of 66.3%among pregnant women. The marked variations in prevalence across regions and countries could be related to geographical factors-climate (rainfall, temperature). For example, it is thought that heat and humidity are important factors favoring the conservation of oocysts in t he soil and thus participate maintaining a high prevalence. It is also known that this difference in prevalence may also be related to differences in dietary habits (Cenci-Goga *et al*; 2011). Data from several African countries suggest that the prevalence of toxoplasmosis during pregnancy varies from one country to another. For example, a seroprevalence rate of 60% and 43.7% were reported in Côte d'Ivoire and Nigeria respectively (Adou- Bryn, 2004; Olusi *et al*; 1996). In Senegal, Ndiaye, *et al;* (2011) reported34.5% of *Toxoplasma* seroprevalence. Morocco, El Mansouri, *et al;* (2007) reported a toxoplaasmosis seroprevalence rate of 50.6%. *T. gondii* prevalence also can be heterogeneous within the same country. For example, a study by Jones and others, (2001) showed that the seroprevalence in the USA varied from 29.2% in the northeast to 20.5% and17.5% in the south-mid west and west respectively. Likewise, the prevalence of toxoplasmosis seems to vary widely among HIV-positive and HIV negative adults. For example, a study conducted in Zambia on HIV –positive and HIV –negative adults described a sero-prevalence of 7% (Zumla *et al*; 1991), while another study on similar group in Ethiopiain 1991 showed a seroprevalence of 80 % (Woldemichael *et al*; 1998).

The reason for this may not be clear but could include several factors such as socioeconomic status, education, sanitary conditions and differences in

dietary habits. There is limited historical information about the disease in Rwanda, in a study done in two rural communities' N genda and N yarutovu now in Bugesera district eastern province, 50% of the adults in both communities had antibodies to *T. gondii*. (Gascon *et al*; 1989).

In Germany the seroprevalence of *T. gondii* correlates with the age of the individuals, since about 20% of the 20 year olds, 30% of the 30 year olds and so on are infected with the parasite (Gross,1994). In immunocompetent individuals, *T. gondii* most often does not cause any or only mild symptoms. However, reactivation of persisting bradyzoites in immunocompromised individuals, i.e. AIDS-patients or patients under immunosuppressive therapy, can lead to life-threatening toxoplasmosis with encephalitis, CNS lesions or chorioretinitis (Ambroise-Thomas *et al*; 1993 and Ferreira *et al*; 2002). This indicates the medical importance of *T. gondii* as an opportunistic pathogen. *T. gondii* is also of major medical relevance during congenital toxoplasmosis acquired after primary maternal infection during pregnancy. This is due to the ability of *T. gondii* to transmigrate through the placenta. Fast replicating parasites can severely injure the fetus eventually leading to fatal toxoplasmosis followed by abortion (Ambroise-Thomas *et al*; 1993). Furthermore, development of the parasite to bradyzoites possibly results in various CNS lesions and/or chorioretinitis. Most often, however, the parasite forms tissue cysts early after transmission resulting in an asymptomatic toxoplasmosis at birth that may reactivate year's later (Ambroise-Thomas *et al*; 1993). Astudy in Iraq showed that the high prevalence of toxoplas- mosis among the investigated high risk women at Al- Hawija and at Al-Baiji districts was due to many risk factors including age, number of deliveries, contact with host animals (small ruminants), contact with uncooked meat,

drinking raw sheep or goat milk as well as neglegable studies on the disease concerned, bad health education, no efficient medication, no surveys and possible environmental pollution with the organisms due to situations of sanctions and series of wars that attacked the country as far as this disease was elevated after Iraq occupation with a frequency of infection more than 40%.(Mohemid *et al;* 2013).

2.7 Epidemiology in Sudan:

Toxoplasmosis in human was identified in Sudan when Carter and Fleck in 1966 used the Dye test (DT) and reported the prevalence of 61% in four different states in the country. Another study done in Khartoum State using ELISA IgG in Sudanese pregnant women showed rate of 34.1% (Elnahas et al; 2003). Abdel-Hameed 1991 in Geizera area in central Sudan reported the prevalence of 41.7% using the LAT. Also, there was study done in North Geizera in childbearing age women and the prevalence was 73.1% (Khalil et al; 2009).Another *T.gondii* seroepidemiological study among pregnant women was made by Elnahas, (1997), who detected an obvious increase in IgG seroprevalence status with low levels of education and socioeconomic status. The sociocultural habit of eating raw liver and marrara was found to be an important risk factor for acquiring *T. gondii* infection. Satti, *et al;* (2011) conducted a study to determine risk factors of toxoplasmaosis among pregnant women in Omdurman, Khartoume state, during the period of June 2006 to July 2008 and diagnose toxoplasmosis serologically using ELISA. The study included those 455 pregnant women between 25 – 35 years of ages had prevalence of *T. gondii* of 38.9% and 12.9% for IgG and IgM respectively, and the results of PCR test showed 16.7%.

High prevalence was found among HIV patients (75%), aborters (58.3%), and suspected cases (55.5%) was reported by Khalil, *et al;* (2012). Another report for khalil, *et al;* (2014) showed high prevalence using ELISA IgG (was 73.1%) from women reside in two village. Musa, *et al*; (2014) examined 163 pregnant women, and found that 33 (20.2%) were positive for (IgG) anti-*Toxoplasma* antibodies, while 130 (79.8 %) were seronegative and none of the examined had IgM anti *Toxoplasma* antibodies. The highest rate of the infection (26.7%) was detected among women aged 21-29 years. A study to determine *T. gondii* antibodies rate (IgM, IgG) on renal transplant recipients in Sudan, was conducted by Mosab *et al*; (2015), a total of 84 serum samples were obtained from renal transplant recipients referred from different areas of the country to Sudanese kidney transplanted association hospital in Khartoum-Bahry. Overall seropositivity rate of toxoplasmosis in renal transplant recipients was found to be 40.5% (34/84). The seropositivity rate increased steadily with age. Also It was found that eight (38.1%) of the 21 subjects had undergone kidney transplantation less than one year ago, and twenty six (41.3%) out of 63 cases have had transplantation year or more than one year ago. The overall seropositivity in males 24/58 (41.4%), was higher than in females 10/26 (38.5%). Abdelhakam *et al*; (2016) examined 104 serum samples collected from pregnant and gynecological women in Tendalty, White Nile State. Samples were screened for the parasite using latex agglutination test (antigen, antibody reaction) the overall prevalence of parasite was found to be 38.5%.

2:8 Pathogenesis:

Parasites enter gastrointestinal cells, after their release from oocysts, where they multiply, disrupt cells, and infect contiguous cells (Remington *et al*,

1995). Organisms may spread first to the mesenteric lymph nodes and then to distant organs by invasion of lymphatics and blood stream (John and Petri, 2006). *T. gondii* infects all cell types, and cell invasion occurs as an active process. Then it generates the formation of a parasitophorous vacule which is resistance to immune responce, (Remington *et al.*, 2005).

Active invasion of macrophages by tachyzoites does not initially trigger oxidative killing mechanism (Montoya and Rosso, 2005*).*

The tachyzoites divided by binary fission, forming an intracellular pseudocyst, which distorts the host cell and ultimately leads to cellular disruption (Nash *et al.*, 2005). Damage associated with host immune response (Jones, 2006). An effective immune response significantly reduces the number of tachyzoites in all tissues. In human, clinical disease is normally limited either to immunocompromised individuals or to congenital disease resulting from an acute infection of expectant mother (Black and Boothroyd, 2000).When pregnant woman acquires *T. gondii*, tachyzoites are hematogenously spread to the placenta. The organism then can be transmitted transplacentally directly to the fetus during gestation or at birth (Giannoulis *et al.*, 2008).

Cysts are the source of organisms that cause recrudescent disease in immunocompromised patients or chorioretinitis into the children and adults with congenital toxoplasmosis (Montoya and Remington, 1996; Nash *et al.*, 2005). With tissue cysts, which are characteristic of chronic infection, the other source is the persistent viable tachyzoites within monocytes and macrophages (Mordue and Sibley, 1997; Wu and Garcia, 2005).

2:9 Clinical manifestations:-

2:9:1 Toxoplasmosis in the immunocompetent patients:

The vast majority of cases of *T. gondii* infection in adults and children are asymptomatic (Remington *et al*., 2005) .Lymphadenopathy is the most common manifestation in the (10%-20%) of otherwise immunocompetent individuals whose primary *T. gondii* infection is symptomatic. (Montoya and Remington, 2000). The clinical manifestations among this group are self limited after weeks to months, about (80-90%) asymptomatic (Schmidt, *et a;l* 2005) Lymphadenopathy may be present, maculopapular rash, fatigue, weakness, pharyngitis, malaise, sore throat, hepatosplenomegaly, myalgias, and a typical lymphocytosis.(Montoya and Remington, 1996; Tenter *et al.*, 2000 and Cox and John, 2005).

2:9:2 Toxoplasmosis in the immunodeficient patients:

In contrast to the relatively favourable course of toxoplasmosis in almost all immunocompetent individuals, immunologically impaired patients usually develop a dreadful and often life-threatening disease (Pappas and Wardrop, 2004). Immunocompromised patients at higher risk for toxoplasmosis include those with hematologic malignancies (particularly patients with lymphoma), bone marrow transplant, solid organ transplant (including heart, lung, liver, or kidney), or AIDS patients. Toxoplasmic encephalitis is the most common presentation of toxoplasmosis in immunocompromised patients (Remington *et al.*, 1983), and the most frequent cause of focal CNS lesions in AIDS patients (Corlos *et al.*, 2007).

It is unclear whether *T. gondii* penetrates the brain more easily than other organs or whether it is more difficult for the brain, as an immunologically privileged site, to eradicate the organism during the initial acute infection and once residual infection has been established (Montoya, 2002).A wide range of clinical findings, including altered mental state, seizures, weakness, cranial nerve disturbances, sensory abnormalities, cerebellar signs, meningismus, movement disorders, and neuropsychiatric manifestations are observed in patients with toxoplasmic encephalitis (Liesenfeld *et al;* 1997). Other organs commonly involved in immunocompromised patients with toxoplasmosis are the lungs, eyes, and heart. (Joseph *et al*; 2010). In the vast majority of immunocompromised patients, toxoplasmosis results from reactivation of a latent infection. In contrast, in heart transplant patients and in a small number of other immunocompromised patients, the highest risk of developing disease is in the setting of primary infection (i.e., a seronegative recipient who acquires the parasite from a seropositive donor via a graft) (Joseph *et al;* 2010).

2:9:3 Congenital toxoplasmosis:

Congenital toxoplasmosis occurs when *T.gondii*, reaches the fetus transplacentally. More than 90% of pregnant women who acquire a primary infection during gestation are asymptomatic (Montoya and Rosso, 2005). If she is infected before pregnancy, the organism will be in the cyst form and there will be no trophozoites to pass through placenta. The mother who is reinfected during pregnancy but who has immunity from a previous infection may not transmit the organism to her child (Warren, 2006). The incidence of fetal infection and damage leading to abortion stillbirth or disease in the newborn (Fihisetti and Gandolfi, 2004). Chance of infecting their fetus is

more in the last trimester, than the first and second ones (Ho-yen *et al.*, 1992; Black and Boothroyed, 2000). Clinical feature of congenital toxoplasmosis in the infant include convulsion, microcephaly, mental retardation and defective vision. There are often no detectable abnormalities at birth but signs like chorioretinitis, generally appear within few years (Cedirc *et al.*, 2004). In 2003, Torrey published a review of literature, reporting that almost all the studies had found that schizophrenics have elevated rates of *Toxoplasma* infection. Acute *Toxoplasma* infection sometimes leads to psychotic symptoms not unlike schizophrenia (Jaroslav, 2007).

2:9:4 Ocular toxoplasmosis:

Ocular toxoplasmosis leads to permanent loss of vision in affected eyes in nearly 25 % of patients (Bhopale, 2003). Ocular disease occurs in a large percentage of congenitally infected patients, but the classical explanation that most cases were due to congenital infection has been challenged (Gilbert and Stanford, 2000). At birth, infected infants may have ocular disease broadly defined as retinochoroiditis or inflammation of the retina and choroid with associated vitritis (Mcleod *et al*; 2006). New ocular lesions can occur at any age after birth in untreated and some treated children. Whilst severity of disease is influenced by trimester in which infection is acquired from the mother (Lihleh *et al*., 2010). Presumed ocular toxoplasmosis was diagnosed on the basis of clinical appearance of retinochoroiditis and a positive serology. Lesions were presumed to be active in the presence of whitish edematous areas (Yan, 2009).

Epidemiological data have shown that most cases of ocular toxoplasmosis result from reactivation of latent infection and not from primary infection.

Disease evolution depends on many factors: the immune response of the host, the virulence of the parasite and environmental factors. Ocular toxoplasmosis can heals spontaneously after two to three months even in the absence of therapy. A review of ophthalmic literature shows that no standard therapy could be proven by large multicentre clinical trials. (Silveira, *et al*., 2002).

2:10 Immune Responses:

A well-orchestrated and effective systemic immune response is responsible for the early disappearance of *T. gondii* from peripheral blood during the acute infection and limits the burden in other organs (Mordue and Sibley, 1997). *T. gondii* induce a strong cellular and humoral response in infected host (Lori *et al*., 2002).

2:10:1 Cellular Immunity:

The importance of cellular immunity against *T. gondii* is shown by the high incidence of toxoplasmosis in the human immunodeficience virus-infected population before high active antiretro-virus therapy was introduced (Luft and Remington, 1992). The cellular immunity is a major factor responsible for resistance against this parasite; cellular immune response plays an active role in driving encystment of the parasite (Charles, 2009). Both immune CD4+ and CD8+ cells can lyse *T. gondii* infected cells (Montoya and Remington, 1996). These T-cell subsets synergize with macrophages, natural killer (NK) and lymphokine- activated killer cells in protective mechanisms (Corry *et al*., 2009). A rapid and remarkable α and β T-cell response plays an important role in the early events of the immune response against the parasite (Peterson *et al*., 2007).Cytokines play a critical role in defense against the infection and are important in the pathogenesis of toxoplasmosis and toxoplasmic

encephalitis (Bonfioli and Orefice, 2005). Following sensitinzation with *T. gondii* the macrophages produce Interleukin - 12 (1L – 12), which together with Interleukin 13 (1L- 13), Interleukin 18 (1L- 18), and Tumor necrosis factor (TNF- α) stimulate NK cells to produce interferon Gamma IFN-γ. IFN-γ acts in synergy with the TNF- α, which induce in the production of nitric oxide which kills intracellular *T.gondii* (Corry *et al* .,2009). Thus, cytokines such as IFN-γ and TNF which activated macrophage function are important for controlling tachyzoites replication during both acute and chronic phases of infection. T- Lymphocytes are the dominant sources of this cytokines during the chronic phase (David *et al*., 2008). Both astrocytes and microglia likely play important roles in the immune response against *T. gondii* within the CNS, whereas *T. gondii* can invade, survive, and multiply within astrocytes, they are killed by activated microglia (Peterson *et al.*, 2007).

2:10:2 Humoral Immunity:

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 antibodie were no longer capable to enter various cells and were readily engulfed by macrophages (Nash *et al.*, 2005). *T.gondii* IgG antibodies when elevated indicate past infection, although elevated *Toxoplasma*-specific IgG levels may last for many years after primary infection (Nazan, 2008).

2. 11 cytokines:

Cytokines are soluble mediators secreted by the cells without any specificity for antigens and which exert their biological action at very low concentration. Their action is essentially local and leads to a modification in cell activity due to paracrine and autocrine effects (Dworkin *et al;* 2002). In the event of toxoplasmosis, they can be divided into 2 main types - protective and regulatory cytokines - without taking into account their strict classification into type-1 or type-2 cytokines. (Rytel, 1966).

2.11.1 Protective cytokine:

Interferon γ (IFN-γ) has numerous biological activities, including: activation of macrophages and NK cells, induction of MHC class-II antigens and inhibition of type-2 cell response. NK cells and TL (CD4+ and CD8+) are the main sources of IFN-γ. Identified back in 1966, in the peritoneal fluid and serum of mice infected with the virulent RH strain of T.gondii, IFN-γ was the first cytokine implicated in resistance to *T. gondii* and remains the keystone of protective immunity to *Toxoplasma* (Rytel, 1966; Suzuki *et al;* 1988). IFN-γ is produced during *Toxoplasma* infection both in sensitive mice and in mice resistant to infection (Suzuki *et al;* 1999). The production of IFN-γ is also found in humans in acute toxoplasmosis and in newborn babies infected during pregnancy, with a correlation between the degree of foetal infection

and the quantity of IFN-γ secreted (Raymond *et al;* 1990) .In mice, the secretion of INF-γ increases the phagocyte activity of macrophages and the cytotoxic activity of CD8+ TL (Ely *et al;* 1999). However, IFN-γ triggers the conversion of tachyzoites into bradyzoites (Jones *et al;* 1985) (Bohne *et al;* 1993; Soete, *et al;* 1994) .At the same time preventing their rupture(Suzuki, *et al; 1988).*A high level of IFN-γ production is strongly correlated with virulent type-1 strains and increased apoptosis (Gavrilescu and Denkers, 2001). Also with intestinal immunopathological phenomena in C57BL/6 sensitive mice (Liesenfeld, *et al;* 1996; Suzuki *et al;* 2000). Interleukin 12 (IL-12), which is secreted by the macrophages and the dendritic cells during antigen stimulation, appears to play a major anti-*Toxoplasma* role during the acute phase of the infection. Indeed, it activates the production of IFN-γ by NK cells and CD4+, CD8+ TL (Hunter *et al;* 1995) The administration of IL-12 combined with the recombinant *T. gondii* SAG (surface antigen 1) surface protein directs the immune response towards a predominantly type-1 profile, associated with high IFN-γ production. This directing of the immune response is linked to a reduction in cerebral parasite load (Letscher-Bru, *et al;* 1998). IL-12 is also essential during the chronic phase of the infection, when it is responsible for maintaining a long-term immune response (Yap *et al;* 2000).The positive regulation of IL-12 is obtained via CCR5-type receptors (Aliberti *et al;* 2000).where as negative regulation is obtained via lipoxins A. (Aliberti *et al;* 2002).

TFN-α is produced by monocyte macrophages, TL and basophil, mastocytes. It exerts an early protective effect by increasing the microbicidal capacities of the macrophages and inducing the secretion of IFN-γ by the NK cell. A pyrogenic factor, TNF-α is liable to induce the secretion of acute

inflammatory phase proteins via the production of IL-6 (Marshall *et al;* 1999). In toxoplasmosis, TNF-α would appear to be essential for macrophage activation and inhibition of parasite replication, but this action can only be exerted in synergy with IFN-γ. This protective action is exerted in mice in both the acute and chronic phase of the disease (Changm *et al;* 1990; Langermans *et al;* 2001 and Johnson, 1992). In addition TNF-α - like IL-12, another monocyte macrophage product, stimulates the production of IFN-γ by NK cells (Tripp *et al;* 1993;Sher *et al*; 1993). Which play a crucial role in the early non-specific response during toxoplasmosis. However, the role of TNF-α in toxoplasmosis is still debated. Some authors report a link between TNF-α and fatal infection in mice and with a harmful cerebral and hepatic action (Black, *et al;* 1989; Beaman and Remington, 1992).TNF-α may aid the intracerebral dissemination of *T. gondii* in mice (Grau, *et al;* 1992).And may be increased in toxoplasmic chorioretintis during primo-infection in human (yamamoto *et al;* 1991) .

Interleukin 6 (IL-6) is produced by a large number of cells, including monocyte macrophages, endothelial cells, fibroblasts, myelomatous and neoplastic cells. The main mediator responsible for hepatocytic production of acute inflammatory phase proteins, it exerts a synergetic action with IL-1, TNF-α and glucocorticoids. IL-6 is therefore a pyrogenic factor and a remarkable stress marker. IL-6 increases the cytotoxic activity of NK cells and later induces differentiation of BL into antibody secreting cells and differentiation of cytotoxic TL. In murinen toxoplasmosis, a gradual increase in serum IL-6 is correlated with clinical signs (Beaman, *et al;* 1992). The administration of an anti-IL-6 monoclonal antibody in a model of murine toxoplasmic encephalitis reduces the inflammatory lesions and number of

cysts in the brains of these mice (Suzuki *et al;* 1994). In ocular toxoplasmosis in IL-6 -/- mice, IL-6 has a protective role (Lyons, *et al;* 2001).However; other reports are contradictory. According to Beaman, *et al; (*1994), IL-6 appears to promote the intracellular multiplication of *T. gondii* in mice. Whereas for other authors, human monocyte macrophages or cells derived from a human astrocytoma do not secrete IL-6 *in vitro* in response to *T.gondii* infection (Friedland *et al;* 1993 and Pelloux *et al;* 1994).

Interleukin 5 (IL-5) is produced by numerous cells (T lymphocyte, mastocytes and eosinophils). IL-5 triggers the growth, differentiation, activation and chemotaxis of eosinophils. It is surprising to observe that in toxoplasmosis, this cytokine is capable of increasing the production of IL-12 and of inducing a certain protection in mice against *T.gondii* infection (Zhang and Denkers, 1999).Nickdel *et al,* 2001 attributed a pathogenic role to it, through an increase in intestinal necrosis. The presence of eosinophils in human congenital toxoplasmosis is probably related to the production of IL-5. (Arnaud, 1975 and Nakazaki *et al;* 2000).

Interleukin 15 (IL-15) is a pleiotropic cytokine secreted by various cells, including macrophages (Doherty *et al;* 1996). It activates the production of IFN-γ in experimentally-induced infection with *T. gondii* (Lee *et al,* 1999). Interleukin 18 (IL-18) is another pleiotropic cytokine produced in a non-specific manner during an inflammatory syndrome. It has the capacity to increase the activity of NK cells in experimentally-induced toxoplasmosis and requires a STAT 4-type transcription factor (Cai, *et al;* 2000).

Interleukin 2 (IL-2) is produced exclusively by the CD4+ TL. In murine toxoplasmosis models, IL-2 has been shown to be protective. The

administration of recombinant IL-2 in mice sensitive to *Toxoplasma* infection leads to an increase in the survival of the animals and a reduction in the number of cysts present in the brain. A parallel increase in lytic activity of the macrophages against *Toxoplasma* and of NK cell activity is also observed. (Sharma *et al;* 1985; Subauste *et al;* 1991 and Denkers *et al;* 1996).

2.11.2 Regulatory cytokines:

Interleukin 4 (IL-4) is secreted by a small number of cells and, more specifically, by type-2 CD4+ TL. Basophils, mastocytes and certain CD8+ TL can also be a source of IL-4. A factor for the activation and differentiation of TL and B lymphocytes (BL), it increases the expression of class-II MHC antigens and triggers IgE isotype switching. IL-4 alone does not appear to influence the intracellular growth of *Toxoplasma in vitro (*Appelberg *et al;* 1992. However, *in vivo* in mice, endogenous IL-4 appears to play an important role in resistance to *Toxoplasma* infection (Villard *et al;* 1995). But it is believed that it may play an immunosuppressant role promoting the passage of *Toxoplasma* through the placenta in mice (Thouvenin *et al;* 1997 and Alexander, *et al;* 1998).

Interleukin 10 (IL10) is secreted by type-2 CD4+ TL, macrophages and certain BL. IL-10 inhibits the proliferation of type-Th1 CD4+ TL, along with the secretion of cytokines by these same cells. It also inhibits the production of nitrate and oxygenated derivatives and of pro-inflammatory cytokines (IL-1, IL-6, TNF-α) by monocyte macrophages. In toxoplasmosis, the *in vivo* administration of an anti-IL- 10 monoclonal antibody to SCID mice delays the death of the animals following *Toxoplasma* infection (Hunter *et al*, 1993) *In vitro,* recombinant IL-10 has immunosuppressant properties on the

proliferation of spleen cells taken from mice infected with *T. gondii (*Candolfi *et al; 1995),* and inhibits the capacity of murine macrophages, activated by IFN-γ, to destroy *T. gondii (*Gazzinelli *et al*, 1992). IL-10 is therefore necessary for the negative regulation of a type-1 intestinal response that may be harmful in C57BL/6 susceptible mice. IL-10 counters the harmful effect of an exaggerated type-1 inflammatory response based on the high production of TFN-α, IFN-γ and NO associated with the intestinal proliferation of *Toxoplasma. gondii. (*Liesenfeld,1999; Villegas *et al*; 2000 and Suzukiy *et al;* 2000).

Transforming growth factor-β (TGF-β) is well known for its immunosuppressant action on leukocyte cell lines. It is considered to be an antagonist of TNF-α, IFN-γ, TNF-β QA and IL-2 (Hunter etal,1995) Langermans *et al;* 2001). The anti-inflammatory action of this cytokine makes it possible to control the development of immunopathological phenomena related to a type-1 immune response in the intestines (Buzoni-Gatel *et al;* 2001), or the brain (Schluter *et al;* 1998). However, TGF-β increases replication of *Toxoplasma* on cultured retinal cells, suggesting that this cytokine may be involved in immunopathological phenomena (Nagineni *et al;* 2002).

2.12 Role of Interleukin 8 (IL8) in toxoplasmosis:

IL-8 is secreted by multiple cell types, including monocytes, neutrophils, epithelial, fibroblast, endothelial, mesothelial, and tumor cells. It is released from several cell types in response to an inflammatory stimulus (Matsushima and Oppenheim; 1989). Chemotaxsis in its target cells like neutrophil and

granulocyte. IL8 has an important role in the innate immune response .It is often associated with inflammation. It has been ciliated as proinflammatory mediator in toxoplasmosis. 1It is well recognized that T cell mediated immunity plays a central role in the host response to intracellular pathogens. Th2 cell mediated immunity and activated macrophage have been shown to play important roles in resistance to cell mediated immunity in *T.gondii* infection. Increased level of IL8 correlate with early acute inflammation or with a reactive form of toxoplasmosis. Interlukein 8 is responsible for activation and recirculation of neutrophils where neutrophils can phagocytose and kill or inhibit tachyzoite of *Toxoplasma*, and showed that human intestinal epithelial cells infected with *T.gondii* elicit rapid secretion of IL8, so it has an important role in innate immunity in response to *Toxoplasma*. Most pregnant with acute acquired infection do not experience obvious symptoms or signs. Acute and latent *T.gondii* Infections during pregnancy are most commonly diagnosed by detecting the immunoglobulin IgG –IgM antibody in the serum samples of the patients using ELISA. Normal pregnancy was accompanied by a reduction in Th-1 productive capacity together with an rising in Th-2 production, most markedly in the third trimester, The cause of repeated pregnancy loss (three or more successive spontaneous miscarriages) are unsolved in the majority of women and it is thought that anomalies in the immune system may have a role in idiopathic recurrent abortion (Makhseed *et al;* 2001). Cellular immune effector mechanisms have been suggested as being responsible for at least a percentage of repeated spontaneous abortion (RSA). Attention has focused on clarifying the immunobiological roles of cytokines in normal human pregnancy following the collected reports of complex cytokine activity within uteroplacental tissue (Michimata *et al;* 2003). Cytokines are important mediators in the bi-directional interaction

between the maternal immune system and the reproductive system during pregnancy (Vesce *etal;* 2002) and (Mohammed *et al;* 2012).

Endometrium also produces IL-8 that is abortogenic. The endometrium, myometrium, and outer decidua contain mast cells that are increased by more than 10-fold in decidua in abortions. Mast cells are essential for inflammation by liberating several multifunctional cytokines including IL-8. The fact that Interleukin-8 secretion is increased by oxidative stress, which in that way causes the enrolment of inflammatory cells, induces a further increase inoxidative stressmediators, making it a key parameter in localized inflammation. If a pregnant mother has high levels of interleukin-8, there is an increased risk of schizophrenia in her offspring (Mekori and Metcalfe, 2000).

Evidence that prenatal infection plays a role in schizophrenia is increasing (Brown and Susser, 2002).The higher levels of second-trimester IL-8 are associated with a greater risk of schizophrenia. There are two possible explanations for this finding. First, in utero infection may have led to the elevation of IL-8 in mothers of subjects who developed schizophrenia spectrum disorders. Second, a noninfectious exposure associated with a proinflammatory cytokine response may have been responsible.(Alan and Brown *et al;* 2004), also infection with the protozoan parasite *T. gondii* has been found to be a relatively strong risk factor for schizophrenia.This association is based on an increased incidence of schizophrenia in individuals with antibodies to *T. gondii* .(Torrey *et al;* 2007). *T. gondii* expresses two tyrosine hydroxylases which can convert tyrosine to DOPA, the rate limiting step in dopamine synthesis. (Prandovszky *et al;* 2011). This increase in dopamine synthesis has been proposed as the mechanism through which *T.*

gondii infection contributes to an increased risk for schizophrenia. However, *T. gondii* cysts that form in brain tissue also produce inflammation. (Hermes *et al;* 2008).

2.13 Role of interleukin 17 (IL17) in toxoplasmosis:

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 IL17 was 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). The role of IL-17 in toxoplasmosis: the initial innate immune response led is neutrophil has also been reported to be critical for successful resolution of the inection. (Gazzinelli *et al;* 1993; Sayles *et al;* 1996 and Bliss *et al*; 2001). Several studies have shown that their loss lead to exacerbation of infection. (Ye *et al;* 2001; Kelly *et al;* 2005), the early induction of neutrophil induction during *T.gondii* infection is dependent on IL-17 mediated signaling. 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 lead to increased mortality. (Yisong and Richard, 2009).

2.14 Diagnosis

2.14.1 Traditional, non-DNA-based diagnostic methods

1.14.1.1 Microscopic diagnosis

The detection of *T. gondii* in fecal, water, environmental and tissue samples has traditionally relied on microscope examination. However, identification based on light microscopy alone is less sensitive and unreliable. The oocysts in fecal, water and environment can be enriched from large volumes of samples by filtration or centrifugation for examination, and the tissue cysts can be stained, which aid in distinguishing the parasites from host cells (Hutchison *et al;* 1979). Giemsa and Haematoxylin and Eosin (HE) staining is simple and cost-effective, and commonly used for this purpose (Gordon *et al;* 1993; Dubey *et al;* 1993).Periodic acid schiff (PAS) can stain amylopection granules in bradyzoites (da Silva *et al;* 2010). These methods are relatively time consuming and require considerable skill to obtain reliable detection results. Electron microscopy was also employed to detect tissue cysts in mouse brain and oocysts in the small intestine of infected cats, but it is difficult to be applicable for routine use (Sims *et al;* 1989).

2.14.1.2 Bioassay

The isolation of *T. gondii* by bioassay using laboratory animals is generally considered as the gold standard for detection of *T. gondii* infection. Secretions, excretions, body fluids, lymph nodes, muscle and brain tissues are possible specimens used for the isolation (Dubey *et al;* 2004). Cats can be used to detect small number of viable *T. gondii* in meat because larger volumes of tissues can be fed to cats, therefore increasing the sensitivity. Overall, the bioassay is expensive and time-consuming (usually requires

6 weeks). Thus, it cannot be used for large-scale screening *(*Dubey *et al;* 2013).

2.14.1.3 Dye test (DT)

The dye test was first developed by Sabin and Feldman in 1948, it has been considered as gold standard for the detection of anti-*T. gondii* antibodies in humans(Sabin *et al ;* 1948 ; Reiter-Owona , *et al;* 1999). DT is both specific and sensitive in humans, but may be unreliable in cattle and avian species (Dube*y et al;* 1985).The major disadvantage of DT requires live parasites and human serum as accessory factors, severely limiting the availability of the DT (Ashbur*n et al*, 2001). The test is potentially hazardous, and requires a high degree of technical expertise, thus only performed in reference laboratories. Though tachyzoites prepared from cell culture can be routinely used in DT, the false negative results may occur in some cases. Therefore, tachyzoites prepared from mice are preferred for DT (Udonsom *et al;* 2010).

2.14.1.4 Modified agglutination test (MAT)

This test was first described by Fulton and Turk *(*Fulton *et al;* 1959). With low specificity and sensitivity, due to the binding of normal IgM to the surface of the parasite and improved by preparing the antigen using a buffer containing 2-mercaptoethanol to remove non-specific IgM *(*Macri *et al;* 2009) . This test detects IgG antibodies, without limitation of host species, but the false negative results may occur during early stages of acute infection. The specificity and sensitivity of MAT are comparable to the DT in most species, but it can produce high false negative results in dogs *(*Zhu *et al;* 2012).The test is very useful in diagnosis of toxoplasmosis in AIDS patients, and acute

glandular toxoplasmosis **(**Montoya *et al;* 2007). In addition, MAT can also be used to detect cardiac fluids for the survey of *T. gondii* infection in slaughtered sheep for human consumption, with higher sensitivity than other serological tests. MAT is so simple and accurate, that is convenient both for laboratory diagnosis and for epidemiological survey (Villena *et al;* 2012).

2.14.1.5 Latex agglutination test (LAT)

In this test, soluble antigen is coated on latex particles, and agglutination is observed when the positive serum is added. LAT is rapid and easy to perform to detect anti-*T. gondii* IgG antibodies **(**Mazumder *et al;*1988). LAT has a sensitivity of 86–94 % and specificity of 100 % in humans, a low sensitivity of 78.6 % and specificity of 61.9 % in sheep **(**Oncel *et al;* 2005). Thus, LAT is often used as a screening tool in epidemiologic survey due to the simplicity of performance, but the positive result requires further examination using other serological tests **(**Holliman *et al;* 1990*)* .LAT has also been modified to detect anti-*T. gondii* IgM antibodies in humans for diagnosis of recent infection.(Sato *et al;* 1987).

2.14.1.6 Indirect hemagglutination test (IHA)

The principle of IHA is that the tanned red blood cells sensitized with *T. gondii* soluble antigen can be agglutinated by the positive serum (Dubey, 2010).However, detectable IHA IgG antibodies are later than DT, so acute and congenital infections are likely to be missed by this test (Eissa *et al;*1990; Balfour *et al;* 1980. The IgG-IHA test is simple and rapid, thus recommended for mass screening in epidemiologic surveys (Caruana, 1980). Described a modified IgM-IHA test by stabilized human red cells coated with a *T. gondii*

heat-stable alkaline-solubilized extract, which can be used for the serodiagnosis of acute toxoplasmosis in humans, with a sensitivity of 100 % and specificity of 98.5 %.(Yamamoto *et al*; 1991).

2.14.1.7 Indirect fluorescent antibody test (IFAT)

IFAT is a simple test detecting both IgG and IgM antibodies, and has been widely used in detection of *T. gondii* antibodies in humans and animals *(*Arthur *et al*; *1988).* Killed *T. gondii* tachyzoites are incubated with test serum, the fluorescent anti-species antibodies are added, and the result is read under a fluorescence microscope. The test shows sensitivities of 80.4–100 % and specificities of 91.4–95.8 % *(*dos Santos *et al;* 2010).Fluorescent-labeled antibodies for a variety of species are commercially available, and the method is relatively inexpensive (Filice *et al; 1983).*A major advantage of IFAT is that the tachyzoites are killed; therefore it is a safe and easy test to perform while still providing reliable results (Özkan *et al;* 2008). It is not impacted by hemolysis (Miller *et al;* 2002), allows for screening of both IgG and IgM antibodies in serum and can be performed using only one dilution while still obtaining accurate results (Seefeldt *et al;* 1989). However, IFAT may be slightly more complicated to run compared to other serologic tests (Shaapan *et al.,* 2008). Additionally, it requires the use of fluorescent labeled conjugates specific to each species tested and specialized equipment fluorescent microscope (Rodrigues *et al;* 2009). Furthermore, this serologic test does not perform well in cats that are co-infected with feline immunodeficiency virus and *T. gondii* (Sucilathangam *et al;* 2010), and at low dilutions, the results may be more difficult to interpret due to background fluorescence (Dabritz *et*

al; 2007). The interpretation of the results is subjective (Seefeldt *et al;* 1989) however, by using only one reader this error is decreased (Miller *et al;* 2002).

2.14.1.8 Enzyme-linked immunosbsorbent assay (ELISA)

The ELISA system usually includes the solid phase antigen or antibody, enzyme labeled antigen or antibody, and the substrate of the enzyme reaction, which can be modified to test both antibodies and antigens .ELISA can be automated so that a large number of samples can be simultaneously tested. There have been different types of ELISA developed to detect *T. gondii* antibodies or antigens, such as indirect ELISA, and sandwich ELISA. An advantage of the ELISA is the potential to obtain objective quantitative results by determining the optical density of the serum sample and classifying the results as positive or negative in relation to the optical density of controls (Seefeldt et al., 1989). Also, it is a cost effective test that can be automated to run a large number of samples in a short amount of time (Mainar-Jaime etal, 2007).In addition, this test can be used to detect both IgG and IgM antibodies for *T. gondii* (Seefeldt *et al*; 1989). Unfortunately, a disadvantage of the ELISA is the requirement of standardization of the antigens (Shaapan *et al;* 2008). Additionally, the need of a photometer to evaluate the results might increase costs (Seefeldt *et al;* 1989) and without a photometer a reader must quantify the colors used to determine positives or negatives (Dubey and Beattie, 1988), which could lead to variability in results between readers. Also, compared to the MAT and IFAT it requires more time and effort (Seefeldt *et al;* 1989). Furthermore, the ELISA performs poorly in detecting *T. gondii* antibodies in cats co-infected with feline immunodeficiency virus and *T. gondii (*Dabritz *et al;* 2007).

2.14.1.9 Immunosorbent agglutination assay (ISAGA)

In this test, microtiter plates are coated with anti-human IgM antibodies, and the serum sample is added to the wells for 2 h at 37 °C to allow the binding of IgM. The specific IgM in serum sample will bind to the anti-species IgM and agglutinate fixed parasite antigens, which is observed as that of MAT *(*Desmonts *et al;* 1981). This test is simpler and easier to perform than the IgM-ELISA, but it requires large numbers of *T. gondii* tachyzoites. Thereafter, the IgM-ISAGA is modified by replacing *T. gondii* tacyzoites with latex beads coated with soluble antigens. IgM-ISAGA can be used for the diagnosis of acute acquired and congenital *T. gondii* infection. ***(***Remington *et al;* 1983).

2.14.1.10 Immunochromatographic test (ICT)

The immunochromatographic test is a rapid detection technique in which the colloidal gold-labeled antigen or antibody is used as the tracer, and the cellulose membrane is used as the solid support. (Thobhani *et al;* 2010) (Goni *et al;* 2012). A rapid immunochromatographic strip using colloid gold conjugated anti-excretory/secretory antigens (ESA). IgG antibodies was developed to detect ESA in acute infection of *T. gondii* as early as 2–4 days post-infection, showing high agreement with ELISA in sensitivity and specificity. As ICT is easy, rapid, and convenient to perform, and no special equipment is required, it is suitable for field application *(*Wang *et al;* 2011).

2.14.2 Molecular methods based on detection of parasite nucleic acids

Molecular methods are used in addition to conventional serological methods for the diagnosis of toxoplasmosis. Conventional methods are usually not

misleading, but are limited in prenatal cases or in immunocompromised patients. For example, a mother may be diagnosed accurately by serology that she has had a current infection during pregnancy and so her baby is potentially at risk of congenital infection but the serology results cannot confirm whether the parasite has been transferred to the baby. However, the molecular diagnostic techniques may do so (Saiki *et al;* 1988).

2.14.2.1 Conventional PCR

Due to inherent limitations of traditional diagnostic methods, PCR can be used in addition to serology to diagnose *T. gondii* infection. PCR is an efficient in vitro enzymatic amplification method that allows specific amplification of DNA from minute amounts of starting material in a short time.To achieve high sensitivity, several multicopy targeting genes are usually used for the detection of *T. gondii* in biological samples, including the B1 gene, the 529 bp repeat element and the internal transcribed spacer (ITS-1) or 18S rDNA sequences. The presence of a parasitaemia is seldom detected therefore PCR of blood has a low negative predictive value. Several other single-copy genes, such as SAG1, SAG2, and GRA1, have also been used as PCR targets in some laboratories *(*Saiki *et al;* 1988).

2.14.2.2 Real-time PCR

Real-time PCR can detect low concentrations of target DNA and quantify starting copies of specific template DNA. The amplification product is measured during each cycle using probes or intercalating dyes, and can be quantified by a standard of known concentration (Kasper *et al*; 2009). Real-time PCR has been successfully used to detect *T. gondii* DNA in human blood, cerebrospinal fluid, aqueous humor, amniotic fluid, and other samples

*(*Kompalic-Cristo *et al;* 2007; Nogui et al; 2009).The real-time PCR is also used to evaluate toxoplasmosis progression and treatment efficacy, since it can estimate the intensity of *T. gondii* infection *(*Menotti *et al*; 2003. The real-time PCR assay with the B1 gene is considered as the best-performing technique for diagnosis of congenital toxoplasmosis, compared with conventional PCR and nested-PCR (Teixeira *et al; 2013).* As a rapid closed-tube system, real- time PCR eliminates the possible risk of contamination and produces reproducible quantitative results. Thus it is suitable for standardization (Bretagne *et al;* 2006). Described a sequence-specific magnetic capture method for the isolation of *T.gondii* DNA from large samples of tissue, which can overcome the heterogenous distribution of *T. gondii* tissue cysts, and the small size of the sample (Opsteegh *et al;* 2010). This technique combined with real time PCR can be used in meat samples, and provide an alternative for bioassays to evaluate the burden of *T. gondii* in various tissues of food-borne animals *(*Jurankova *et al;* 2014).

2:15 Treatment

It is important to confirm the presence of the parasite, especially among pregnant women if it is confirmed, treatment should be started to prevent the transmission to the fetus (Jennifer *et al.,* 2003). Drugs used for treatment include:

Pyrimethamine: Widely used for therapy of toxoplasmosis, this drug acts synergistically by blocking the metabolic pathway involving *p*aminobenzoic acid and folic acid cycle respectively (Petersen and Schmidt, 2003). Sometimes thrombocytopenia or leucopenia may develop, although these drugs have a beneficial action when given in the acute stage of the disease,

they will not usually eradicate infection (Mc Cabe, 2001). Pyrimethamine could depress bone marrow threatening the immunocompromised patients and its usage is not recommended during pregnancy especially in the first trimester of gestation due to its potential teratogenic effects (Mui *et al*., 2005). Pyrimethamine usually given in synergistic combination with sulfadia- zine for *Toxoplasma* infection treatment (Mui *et al*., 2005).

Spiramycin: This drug is relatively not toxic to the mother or the fetus. It is concentrated in placental and binds tissues, thus it is used to prevent transmission of *T. gondii* from the mother to the fetus (Jennifer *et al*., 2003).

Clindomycin: This drug is quickly absorbed and diffuses well in the CNS and therefore, it was used as an alternative to sulfadiazine (Cox *et al.*, 2005). It is rarely used to treatment the primary maternal infection in pregnancy or congenital infection because it enters fetal blood when given to pregnant women.A major side effect of clindomycin is ulcerative colitis (Mc Cabe, 2001).

Atovaquone: Many reports showed the efficacy of atovaquone combined with other drugs against murine model of toxoplasmosis (Khan *et al*., 1999). It has been observed the effect of the drug not only against tachyzoites but also against bradyzoite containing *T. gondii* cysts (Remington *et al*., 1995).

Azithromycin: Azithromycin is a semi-synthetic macrolide antibiotic which is effective in prophylaxis and early infection of *T. gondii* (Degerli *et al.*, 2003; Tabbara *et al.*, 2005). This drug has an activity against both tachyzoite and cyst forms of the parasite (Detgerli *et al.*, 2003).

Azithromycin is safely be used both during pregnancy and immunocompromised patients, and that is active in toxoplasmosis located in the CNS (Lode *et al*., 1996).

Changing capsules: The effect of combined traditional Chinese medicine, changing capsule on *T. gondii* tachyzoite was studied by Chinese investigator and found that changing capsule were scientifically better than the current clinical drugs being used (Zhang *et al*., 2006 a). Other ultra structural studies in China by Zhang *et al*. (2006 b) carried out to search for safe and effective drugs to be used for treatment of toxoplasmosis in pregnant women and infants. It was observed that changing capsules were more effective in killing *T. gondii* tachyzoites of infected mice and damaging the ultrastructure of the parasite. (Zhang *et al*; 2006 b).

2.16 vaxcination:

Toxoplasma gondii is ahighly successful parasitic organism that is able to infect and live within a very wide range of different animal hosts. In most immune comptent individuals, infection with *T.gondii* results in development of protective immune response against the disease. In addition, chronically infected women can exhibit adegree of immunity normally sufficient to protect their fetus from conjental infection even if re-exposed during pregnancy. These evidences there fore would suggest that vaxcine against toxoplasmosis has ahigh likelihood of success.although there is currently no licensed *T.gondii* vaxcine available for human, we must remain optimistic that it will be possible to develop an effective and safe vaxcine against toxoplasmosis for animals and humans. (Qi Liu *et al* ;2012)

Chapter three

Materials and methods

Materials and methods

3.1 Study design:

It's a comparative cross-sectional one designed to assess immunoglobulins and cytokines in pregnant women

3.2 Study area and population:

The study was conducted in Khartoum state targeting the pregnant women attending ante-natal clinics and hospitals. The targeted women were categorized according to age groups (15- 20, 21-30, 31-40 and 40-45 year) and stage of pregnancy (1st, 2nd and 3rd trimesters). ,

3.3 Sample size:

The sample size is 300 sera from pregnant women. In naddition to 40 sera from none infected pregnant women as control group as calculated by formula:

$$n = \frac{Z^2\, pq}{l^2}$$

3.4 Period of study:

The study was conducted during the period from April 2015- May2017.

3.5 exclusion criteria: any pregnant women over 45 years and under 15 years must be excluded. Also any one who refused to donate sample was excluded.

3.6 inclusion criteria: any pregnant women in age from 15-40years included in research. Also any one who agreed to donate sample was included.

3.7 Ethical consideration: Ethical clearance obtained from the health authorities in the state. The purpose and procedures of the study was explained to all participants and a written informed consent was obtained from all of them.

3.8 Collection of Samples:

The blood samples were collected from 300 pregnant women under direct medical supervision. Blood samples collected by 5 ml venipuncture into plain tubes, after clotting of blood .Serum was by centrifugation at 5000 rpm for 10 min. Serum samples was kept in -20°C till performance of serological tests.

3.9 Quality control:

The following criteria were used to consider the results as valid:

1. Substrate blank: absorbance value was taken to be less than or equal to 0.100.
2. Negative control: absorbance value was taken to be less than 0.120 after subtracting the blank.
3. Positive control: each of the individual absorbance values was not to differ more than 30% of the mean of the three values.

3.10 Methods:

Serum samples for toxoplasmosis was investigated and screened using the Toxo-latex test, Ict for toxoplasmosis and ELISA and IL-8, IL-17 tested using ELISA.

3.10.1 Toxolatex:

3.10.1.1. Slide method:

Toxo-latex test (Linear Chemicals) is a rapid slide agglutination procedure, developed for the direct detection of anti-*Toxoplasma* antibodies in human serum. The test performed by testing a suspension of latex particles created with antigenic extract of *T. gondii* against unknown samples. The presence or absence of a visible agglutination was indicating the presence or absence of anti-*Toxoplasma* antibodies.

3.10.1.2. Titration method:

For a qualitative test, 50 µl of patient serum was placed into one of the circles on the card. One drop of the positive and negative controls was dispensed into two additional circles. 50 µl of Toxo-latex reagent added to each circle and the contents of each circle was mixed well. The slide was rotated slowly for 5 minutes and observed for agglutination.

For a quantitative test, 50 µl of 0.9% saline solution placed into each of the circles of a card (as diluent). To circle one, 50 µl of serum added to saline and mixed. 50 µl of the mixture transferred to the second circle and the 2-fold serial dilution continued in a similar manner up to the six circles and 50 µl discarded from the last circle. Final sample dilution was: 1:2, 1:4, 1:16, 1:32, 1:64 and 1:128 and the titer determined by the last agglutination.

3.10.2 Toxo IgM\IgG rapid test (cassette)

The foil pouch was removed and it was immediately used. Best results obtained when the assay was performed within one hour. The test was placed

on a clean and level surface. The dropper was held vertically and transferred 3 full drops of serum or plasma (approximately 75ul) to the specimen well of the test device. Air bubbles were avoided. After waiting for colored line to appear, the results were read within 15 minutes.

3.10.3 Enzyme Linked Immuno Sorbant Assay (ELISA)

The original ELISA uses a soluble antigen preparation made from *Toxoplasma* RH strain tachyzoites is layered into wells in a microtitre plate. Test sera will be added, followed by an anti-species enzyme-labelled conjugate such as horseradish peroxidase-labelled anti- -IgG. Attachement causes a colour change in the substrate that is directly related to the amount of bound antibody. It is read with a spectrophotometer at the absorbance specific to the substrate. (Dubey and Beattie, 1988). Defined anti-species conjugates, substrates and whole kits are commercially available.

3.10.3.1 Protocol of Enzyme Linked Immuno Sorbant Assay IgM (ELISA)

100ul of positive and negative controls and diluted patients samples into the individual microplate wells according to the pipetting protocol. They were incubated for 30 minutes at room temperature 18to25c. Washing was done by emptying the wells and subsequently washing for 3times using 300ul of working strength washing buffer. The wash buffer was left in each well30-60 seconds per washing cycle. After washing thoroughly all liquid from the microplate was disposed by tapping it on absorbent paper with the openings facing downwards to remove all residual washing buffers. After that 100ul of enzyme conjugate (peroxidase-lablelled anti human IgM) was pipetted into each of the microplate wells and incubated for 30minutes at room temperature.

The wells were empted again and washed as described beford. After that 100ul of chromogen and substrate solution were pipietted into microplate wells and incubated for 15 minutes at room temperature, and 100ul of stop solution were pipitted into microplate wells in the same order and at the same speed as the chromogen- substrate was introduced. Photometeric measurement of the colour intensity was made at a wavelength of 450nm and refrence wavelength between 620nm and 650nm within 30minutes of adding the stop solution. Prior to measuring the microplate were gently shaken to ensure homogenous distribution of the solution.

The extinction value of the calibrator defines the upper limit of the refrence range of non infected persons cut-off recommended by **Euroimmun. Co.**

3.10.3.2 Protocol of Enzyme Linked Immuno Sorbant Assay IgG (ELISA)

100ul of positive and negative controls and diluted patient samples was transferred into microplate wells according to the pipetting protocol. They were incubated for 30 minutes at room temperature 18to25c.

Washing was done by empty the wells and subsequently wash 3times using 300ul of working strength wash buffer. The wash buffer was left in each well30-60 secondsper washing cycle. After washing thoroughly all liquid from the microplate was disposed by tapping it on absorbent paper with the openings facing downwards to remove all residual washing buffers. After that 100ul of enzyme conjugate was pipetted (peroxidase-lablelled anti human IgG) into each of the microplate wells and incubated for 30minutes at room temperature. The wells were empted again and washed as described beford. After that 100ul of chromogen was pipetted and substrate solution into each

of the microplate wells and incubated for 15 minutes at room temperature. 100ul of stop solution were pipitted into microplate wells in the same order and at the same speed as the chromogen- substrate was introduced. Photometeric measurement of the colour intensity was made at a wavelength of 450nm and refrence wavelength between 620nm and 650nm within 30minutes of adding the stop solution. Prior to measuring the microplate were gently shaken to ensure homogenous distribution of the solution.

The extinction value of the calibrator defines the upper limit of the refrence range of non infected persons cut-off recommended by **Euroimmun. Co.**

3 .10.3.3 Human interleukin 8 (IL8):

Ten wells were set for standard in amicroelisa stripplate. In well 1and 2, 100ul standard solution and 50ul standard dilution buffer were added and mixed well. In well 3 and well4, 100ul solution from well1 and well 2 were added respectively, then 50ul standard dilution buffer were added and mixed well. 50ul solution was discharded from well 3 and well 4 .in well5 and well6, 50ul solution from well 3 and well4 were added respectively. Then 50ul standard dilution buffer were added and mixed well. In well 7 and well 8, 50 ul solutions from well 5 and well 6 were added respectively. Then 50 ul standard dilution buffers were added and mixed well. In well 9 and well 10,50ul solution from well 7 and well 8 were added respectively. Then 50ul standard dilution buffer were added and mixed well. 50ul solution was discharded from well 9 and well 10. After dilution, the total volume in all the wells was 50ul and the concentrations were 180pg\ml, 60pg\ml, 30pg\ml and 15pg\ml, respectively.

In microelisa stripplate, a well was left empty as blank control. In sample wells, 40ul sample dilution buffer and 10ul sample were added (dilution factor is 5).samples was loaded onto the bottom without touching the well wall. Mixed well with gently shaking. Incubated 30 min at 37c after sealed with closure plate membrane. The concentrated wash buffer was diluted with distilled water (30times for 96T and 20 times for 48T).Carefully peel off closure plate membrane, aspirated and refilled with the wash solution. The wash solution was discharged after 30 seconds. The washing procedure was repeated for 5 times. 50 ul HRP-conjgate reagents was added to each well expect the blank control well. And incubated for 30 min at 37c and washed for 5 times. 50ul from chromogen solution A and 50ul chromogen solution B were added to each well, mixed with gently shaking and incubated at 37c for 15 minutes. Avoiding light, 50ul stop solution was added to each well. The absorbance O.D was readed at 450nm using amicrotitre plate reader. The OD value of the blank control well was set as zero.

The extinction value of the calibrator defines the upper limit of the refrence range of non infected persons cut-off recommended by **Sunlong.Co.**

3.10.3.4 Human interleukin 17 (IL17):

Ten wells were set for standard in amicroelisa stripplate. In well 1and 2, 100ul standard solution and 50ul standard dilution buffer were added and mixed well. In well 3 and well 4, 100ul solution from well1 and well 2 were added respectively. Then 50ul standard dilution buffer were added and mixed well. 50ul solution was discarded from well 3 and well 4 .In well5 and well6, 50ul solution from well 3 and well4 were added respectively. Then 50ul standard

dilution buffer were added and mixed well. In well 7 and well 8, 50ul solutions from well 5 and well 6 were added respectively. Then 50ul standard dilution buffers were added and mixed well. In well 9 and well 10, 50ul solution from well 7 and well 8 were added respectively. Then 50ul standard dilution buffer were added and mixed well. 50ul solution was discharged from well 9 and well 10. After dilution, the total volume in all the wells is 50ul and the concentrations are 180pg\ml, 120pg\ml, 60pg\ml, 30pg\ml and 15pg\ml, respectively.

In microelisa stripplate, a well was left empty as blank control. In sample wells, 40ul sample dilution buffer and 10ul sample were added (dilution factor is 5).samples was loaded onto the bottom without touching the wellwall, mixed well with gently shaking. Incubated 30 min at 37c after sealed with closure plate membrane. The concentrated washing buffer was diluted with distilled water (30times for 96T and 20 times for 48T).Carefully peel off closure plate membrane, aspirated and refilled with the wash solution. The wash solution was discharged after resting for 30 seconds. Repeated the washing procedure for 5 times. 50ul HRP-conjugate reagents were added to each well expect the blank control well. And incubated for 30 min at 37c and washed for 5 times. 50ul chromogen solution A and 50ul chromogen solution B were added to each well, mix with gently shaking and incubate at 37c for 15 minutes. 50ul stop solution was added to each well. The absorbance O.D was readed at 450nm using amicrotitre plate reader. The OD value of the blank control well was set as zero.

The extinction value of the calibrator defines the upper limit of the refrence range of non infected persons cut-off recommended by **Sunlong.Co.**

3.11 Data analysis

A statistical software package of SPSS and Exel were used for data analysis, the anti-*Toxoplasma* antibodies and cytokines (IL8 and IL17) were estimated. Data was analyzed to determine the risk factors considered to be of biological importance ($p \leq 0.05$) for association with *T. gondii* infection. .

Chapter four

Results

Results

4.1 Overall prevalence of *T.gondii* by various diagnostic methods:

In this study, 300 blood samples were collected and examined for *T. gondii* infection. The results showed 90(30%) serum samples were positive by the routine latex agglutination test while ICT and ELISA detected 46(15.3%) and 68 (22.6%) positive cases respectively (**Table 4.1).**

Table (4.1): Prevalence of *T. gondii* in study population by various serological methods:

Method	Number of examined samples	Number of positive cases	Percentage (%)	P.value
Latex	**300**	**90**	**30%**	
ELISA		**68**	**22.6%**	
ICT		**46**	**15.3%**	

4.2 Prevalence according to IgG and IgM specific antibody for *T. gondii* using ELISA.

Out of the 300 surveyed pregnant women, 65(21.7%) serum samples were found to have IgG while IgM was showed only 3(1%) IgM. (**Table 4.2).**

Table (4.2): Prevalence according to IgG and IgM specific antibody for *T. gondii* using ELISA.

Type of immunoglobulin by ELISA	Total	Positive cases	Percentage (%)	p.value
IgG	300	65	21.7%	
IgM	300	3	1%	

4.3 Prevalence of *T.gondii* immunoglobulin according to age groups using ELISA:

Surveyed populations were divided into four age groups: (up to 20 years), (21-30), (31-40 years) and (more than 40 years). The positive cases within each age group showed IgM in one (0.3%), one (0.3%), one (0.3%) and 0(0%) respectively. On the other hand IgG was detected in 9(3%), 36(12%), 19(6.3%) and one (0.3%) respectively. (**Table and Fig 4.3**).

Table (4.3): prevalence of *T.gondii* immunoglobulin according to age groups:

Immunoglobulin by ELISA	Age group(years)				Total
	15- 20	21-30	31-40	41-45	
IgM	1	1	1	0	3
IgG	9	36	19	1	65

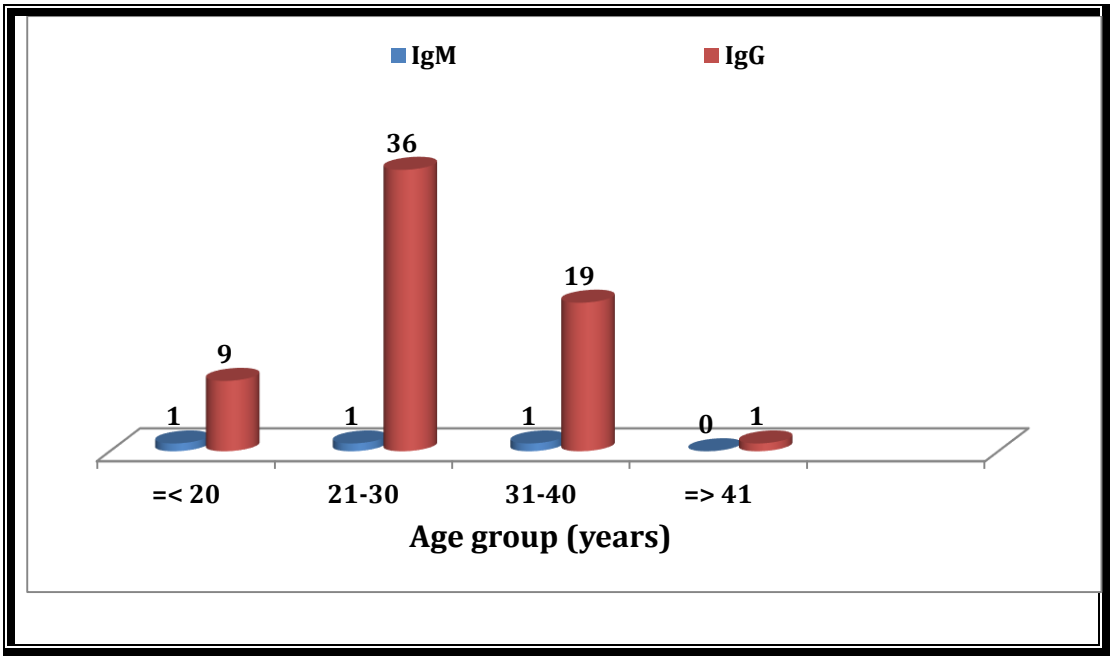

Fig (4.3): Prevalence of *T.gondii* immunoglobulin according to age groups.

4.4 Anti Toxopasmal antibodies (IgM, IgG) in pregnant women who consume raw meat using ELISA:

The result showed that out the 300 pregnant women, 123(41%) were consume raw meat. Among them 32(26%) showed IgG, while one (0.8%) was positive with IgM by ELISA. Also, out of the 300 individuals, 177(59%) not consume raw meat among them, 33(18.6%) and 2(1.1%) showed positive results for IgG and IgM respectively. **(Table 4.4.)**

(**Table 4.4): Anti Toxopasmal antibodies (IgM, IgG) in pregnant women who consume raw meat using ELISA.**

Consuming raw meat	Number examined	IgG positive	IgM positive	p.value
Consume	123	32 (26%)	1 (0.8%)	0.127
Not Consume	177	33 (18.6%)	2 (1.1%)	0.786

4.5 Antitoxoplasma antibodies in pregnant women who had contact with cats:

In pregnant women 153(51%) of those who had some contact with cats, IgG and IgM were found to be 39 (25.5%) and 3 (2%) respectively. In these who had no contact (147women), IgG antibodies was encountered in 26 (17.7%) and no IgM was found in there. In both cases the statistical differences appeared to be insignificant **(Table 4.5).**

Tale 4.5 Antitoxoplasma antibodies in pregnant women who had contact with cats:

Cat contact	Number examined	IgG positive	IgM positive
Yes	153	39(25.5%)	3(2%)
No	147	26(17.7%)	0 (0%)

4.6 The rate of Anti-*Toxoplasma* antibodies according to pregnant women who had history of misscarge:

The results showed that out of 189(63%), who had previous abortion IgG and IgM were found in 42(22.2%) and 3(1.6%) cases respectively. Also out of 111 women who did not witness abortion, 23(20.7%) cases in which IgG detected and no IgM was detected in non aborters. Statistically there is no significant relation. **(Table 4.6)**

Table 4.6: The rate of Anti-*Toxoplasma* antibodies according to pregnant women who had history of misscarge:

Abortion	Number examined	IgG positive	IgM positive	p.value
Yes	189	42(22.2%)	3(1.6%)	0.76
No	111	23(20.7%)	0(0%)	0.18

4.7 The rate of Anti-*Toxoplasma* antibodies according to pregnancy trimesters:

The 300 studied pregnant women were divided into 3 groups according to trimester of pregnancy as first, second, third trimester. Using ELISA IgM, the positive cases was 0, 1 and 2 respectively; while the results of ELISA IgG showed 15, 14, 36 cases respectively **(Table and Fig 4.7).**

Table 4.7: The rate of Anti-*Toxoplasma* antibodies according to pregnancy trimesters:

Pregnancy trimester	IgG	IgM	Total
First trimester	15	0	15
Second trimester	14	1	15
Third trimester	36	2	38
Total	65	3	68

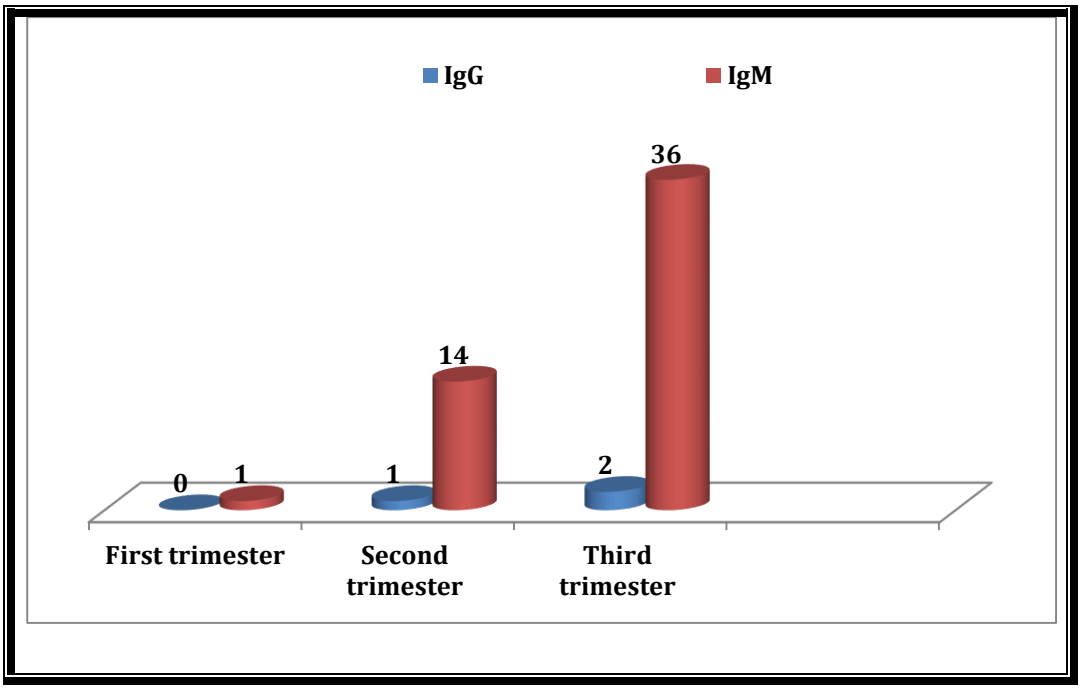

Figure 4.7: The rate of Anti-*Toxoplasma* antibodies according to pregnancy trimesters:

4.8 The rate of cytokines profile (IL8 and IL17) according to history of misscarge:

Within the 189 study population with previous abortion, 12(6.3%) and 13(6.8%) positive cases of IL8 and IL17 respectively. **(Table 4. 8).**

Table 4.8: The rate of cytokines profile (IL8 and IL17) according to history of misscarge:

History of misscarge	Number examined	IL8 positive	IL17 positive
Yes	189	12	13(6.8%)
No	111	15	11(9.9%)

4.9 The rate of cytokines profile (IL8 and IL17) in pregnant women who had toxoplasmosis:

Within the 65 positive cases of ELISA IgG, 16 (24.6%) and 13 (20%) were positive by detection of IL8 and IL17 respectively. The result showed no positive IL8 and IL 17(0%) within the 3 positive cases of ELISA IgM. (P value=0.00 significant **(Table 4.9).**

Table 4.9: The rate of cytokines profile (IL8 and IL17) in pregnant women who had toxoplasmosis:

Type of immunoglobulin	Positive cases	IL8 positive	IL17 positive
IgG	**65**	**16(24.6%)**	**13(20%)**
IgM	**3**	**0(0%)**	**0(0%)**
Total	**68**	**16**	**13**

4.10 Comparison of mean level of cytokines (IL8, IL17) in pregnant women and non infected pregnant women controls:

In non infected pregnant women controls, the mean IL8 serum concentration was 68.9pg\ml while in the pregnant it reaches 210.25 pg\ml. This showed statistical difference (p.value=0.00).Also, in non infected pregnant women controls, the mean IL17 serum concentration was 54.8pg\ml while in the pregnant it reaches 203.15 pg\ml. This showed statistical difference (p.value=0.00). **(Table 4.10).**

Table 4.10: Comparison of mean level of cytokines (IL8, IL17) in pregnant women and non infected pregnant women controls.

Interleukin	Number examined	Positive number	IgM/IgG	Mean of interleukin In pregnant	Mean of control	p.value
IL8	300	27	16	210.25	68.9	0.00
IL17	300	24	13	203.15	54.8	0.00

Discussion

Toxoplasmosis is widely spreading around the world affecting human and animal (Buxton, 1990). Although the first report of human toxoplasmosis in Sudan was done in 1966 by Carter and Fleck, the situation of the disease is not clear and its risk cannot be excluded, Serum testing is still used for diagnosis of toxoplasmosis with IgG antibodies that indicate latent or chronic infection and IgM indicate recent or active infection.

In this study anti *T.gondii* antibodies were screened in 300 sera from pregnant women by three different methods (latex agglutination, ICT for toxoplasmosis and ELISA).The results showed that the overall seroprevalence of toxopasmosis was 30% by latex agglutination test (LAT) in the study population, this rate is rather high which indicate that the individuals were subjected to conductive risk factors, although such factors discussed by authers are not quite confirmed. This result is similar to a previous study done in Philippines in which the prevalence was 27.1 % (Salibay *et al* 2008). However it was lower than that reported by Dumas (1990) who reported 43.6% in Senegal by the LAT, Han *et al*, (2008) in Korea who found only 3.4%. In Sudan, (Abdel-Hameed, 1991) detected prevalence rate of Toxoplasmosis on 41.7% in Gezira state by LAT, Secondly, Elnahas *(*2003) found a rate of 34.1% in Khartoum. Based on these data and the present results it seems to be reasonably to conclude that toxoplasmosis common in pregnant women in Sudan. The difference in these various finding may be attributed to several factors such as personal hygiene, geographical areas, climate, cultural and food habits.

Using enzyme linked immunosorbent assay (ELISA) technique the prevalence in this study was 22.6%. Several studies were done over the world using ELISA IgG, some of them disagree with these results .Some showed a

high prevalence rate than the results obtained for example in Turkey 77% was recorded (Ozcelik *et al*., 1996), and in Togo 75% were reported by Deniau et al, (1991). Also the results obtained by Satti, 2011 showed that the prevalence was 38.9% by ELISA IgG in Khartoum state; also Khalil (2014) was found 73.1% by using ELISA IgG in rural areas in Sudan. It may also disagree with result obtained in 2006 by Maha *et al* who showed that the prevalence using ELISA was 35.1% positive IgG antibodies to *T. gondii* in Sudanese pregnant women. The result however, agreed with Musa (2014) who showed that 20.2% of pregnant women were positive for IgG. Although there is slight variation in the result obtained by Latex and ELISA, they showed anti-*Toxoplasma* antibodies.That means ELISA, not only confirmed Latex results but also showed prevlance of infection.

Using ELISA, the present study confirmed that 65(21.7%) of women had latent toxoplasmosis; this form of toxoplasmosis is generally considered to be asymptomatic, as no clinical signs of infection were observed, this is expected as patient with latent infection are usually asymptomatic. During the latent toxoplasmosis the parasite survives in the dormant form of bradyzoites mostly in the neural and muscular tissue of the host and probably lasts for the whole life of infected person (Remington and Krahenbuhl, 1982) and it can turn into acute toxoplasmosis only after serious violence of integrity of immune system such as AIDS, treatment with immunosuppressive drugs (Mocsny 1992; Heitman and Irizarry, 1997).

When the surveyed women were categorized into 4 groups, the results indicated that the higher rate was encountered in the age group of 21-30 years, followed by the next group 31-40 years. approximately, corresponds with the age group 26-30 years previously reported by Khalil *et al* ;(2013) and disagree with the results revealed by AL-Dahmoshi *et al*; (2013) in Iraq that

the age group ranged from 14-24 years was more infected with toxoplasmosis. The significant effect of age on *T.gondii* serostatus in our study subjects may suggest that the higher age group had long duration of exposure.

Risk factors that most strongly predicted acute infection in pregnant women were eating raw or undercooked lamb, beef and contact with soil. Other factors including drinking unpasteurized milk, and working with animals, contact with cats, cats' faeces were risk factors for infection. Cats are the definitive host where sexual multiplication of *T.gondii* takes place and excrete the oocysts with feces; due to this they are considered the major source of *T.gondii* infection to humans and animals. Exposure to cats has been considered a major risk factor for acquisition of infection (Weigel *et al*., 1999). In the present study, it appears that cats have no direct role in transmission of the disease and there is no stastistically association between cat contact and infection (P= 0.88 and 0.1 with IgM and IgG respectively). However, several studies have concluded that exposure to cats increased the risk of *T.gondii* seropositivity (McCulloch *et al*., 1963; Etheridge and Frenkel, 1995), other studies found no association (Buffolano *etal*., 1966; Fisher and Reid, 1973; Nissapatorn *et al*., 2002; Ertug *et al*., 2005; Alvarado-Esquivel *et al*., 2006). Exposure to cats is not sufficient for transmission of *T. gondii* to women; infection in cats and personal hygiene should be evaluated. In Sudan the contact between people and cats is not as in Europe. The association of cats and human toxoplasmosis is difficult to assess by epidemiological surveys because soil, not the cats, is the main culprit (Ertug *et al*., 2005). Oocysts are not found on cat fur (Dubey, 1995) and are often buried in soil with cat faeces (Dubey, 2000).

The role of consumption of raw meat in the acquisition of *T. gondii* infection has not always been clear. Several studies have found no association (Peterson

et al., 1972; Riemann *et al*., 1975; DiGiacomo *et al*., 1990; Seuri and Koskela, 1992; Weigel *et al*., 1999; Nissapatorn *et al*., 2002; Alvarado-Esquivel *et al*., 2006), although some studies have identified an association between eating raw meat and *T. gondii* seropositivity (Buffolano *et al*., 1966; Konishi and Takahashi, 1987; Cook *et al*., 2000; Alvarado-Esquivel *et al*., 2006). In this study, meat consumption has no role in disease transmission as no statistically association that may due to people recently stop that habbit of eating raw or uncooked meat.

The role of toxoplasmosis in women with history of miscarriage is still unsettled, Women with previous history of miscarriage in the study group showed *T. gondii* sero- prevalence rate of 42 and 3 using ELISA IgG and IgM respectively compared to 23 and 0 among others with no past history of miscarriage with no significant difference between them (P =0.76 and 0.18) respectively. Several studies found no association between *T. gondii* sero-prevalence and history of miscarriage. Similarly result of Al Hindy and Elnahas, (1997) in Khartoum showed no strong association between toxoplasmosis and women with history of miscarriage.

Acute toxoplasmosis causes host cell lysis and an inflammatory infiltrate consisting of lymphocytes, macrophages, and neutrophils. One signal for the observed cellular infiltrate after *T. gondii* infection is the release of pro-inflammatory chemokines from infected cells. Infection of primary fibroblasts, as well as transformed epithelial cell lines, with *T.gondii* stimulates secretion of the proinflammatory chemokines like IL-8. The chemokine response is dependent on invasion by live tachyzoites and subsequent host cell lysis. Furthermore, supernatants or lysates from *T.gondii* infected fibroblasts could elicit significant IL-8 secretion (Denney *et al;* 1999). Increased level of IL-8 correlates with early acute inflammation or with

a reactive form of toxoplasmosis. IL-8 is responsible for activation and recirculation of neutrophils and neutrophils can phagocytose and kill or inhibit tachyzoites of *T. gondii* and showed that human intestinal epithelial cells infected with *T.gondii* elicit rapid secretion of IL-8 (Ju *et al*; 2009), so it has an important role in innate immunity in response to *Toxoplasma*.

In this study, a high significant increase in the mean serum level of IL-8 level in pregnant women with serological evidence of with *T.gondii* (210.25 pg/ml) was statistically significantly higher than in non infected pregnant control women. (68.9 pg/ml p.value =0.00). In this report, the mean level of IL-8 in pregnant women with serological evidence of *T.gondii* infection was higher than other groups. The significance of this finding for the outcome of the pregnancy remains uncertain. Pregnant women produced an endocervical cytokines response that was 2-fold higher than that produced by non pregnant women, also the proinflammatory cytokines milieu in the cervix is enhanced in pregnant women with bacterial vaginosis compared with that in non pregnant women (Richard, *et al;* 2007).

The present study showed a highly significant increase in the mean serum level of IL-17 in patients with IgM\IgG seropositivity (203.15pg/ml) when compared with serum levels in non infected pregnant control group (54.8 pg/ml). The early increase in serum level of IL-17 in the present study match the results of several researchers (Gazzinelli *et al:* 1993) who found that an early increase in IL-17 had been reported in early stage of infection. Ye et al., (Kelly *et al;* 2005) found that IL-17 was involved in the development and early recruitment of neutrophils, which are essential to clear the parasites during initial stages of infection.

Conclusions

-As a general conclusion, it can be stated that the frequency of specific IgM and IgG antibodies using ELISA is considered high among pregnant women investigated for Toxoplasmosis.

- High rate was encountered in the age group of 21-30 years.

- Presence of cat and consumption of meat did not constitute high risk factors.

- A highly significant increase in the mean serum level of IL8 and IL-17 in patients with IgM \ IgG seropositivity, when compared with serum levels in non infected control group.also patient infected with *Toxoplasma gondii* showed increased production of cytokines responsiple for humoral and cellular response.

Recommendation

-Screening of toxoplasmosis during pregnancy using gold standered method as ELISA and Real time PCR.

-Investigation for full blood count to observe leucocytosis specially neutrophils.

- Monitoring of cytokines.

-Avoiding of risk factor to control infection.

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Appendix

Shendi University

College of High Graduated Studies

Faculty of Medical Laboratory Science

Questionnaire NO DATE……..

1- Age.

15-20 ☐ 21-30 ☐ 31-40 ☐ 41-45 ☐

2- Trimester of pregnancy.

First ☐ Second ☐ Third ☐

3- Previous abortion.

yes ☐ No ☐

4- Presence of cat at home

yes ☐ No ☐

5- Consumption of raw meat

yes ☐ No ☐

6- Previous infection with Toxoplasmosis.

yes ☐ No ☐

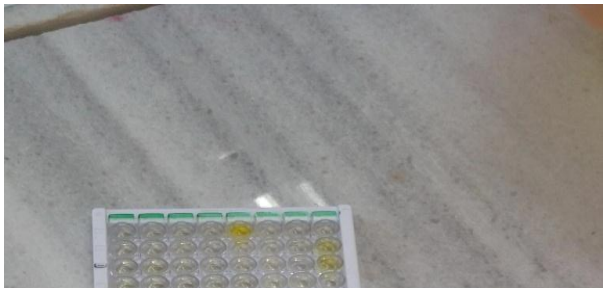

Plate one: ELISA plate.

Plate two: ELISA apperatus