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Prevalence of Toxoplasmosis in cancer patients in Tripoli, Libya

Thesis Submitted to the School of Basic Sciences at the Libyan Academy, in Partial Fulfilment of the Requirements for the Degree of Master of Science of Zoology

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Dedication

To my father, mother, sisters, and my husband

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First and foremost thanks to Almighty Allah who gifted me health, patience and strength to complete this work.

I have to thank my parents for their love and support throughout my life. Thanks also to my sisters, children, husband and the rest of my family.

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LIST OF ABBREVIATION

AIDS	Acquired Immune Deficiency Syndrome
CNS	Central Nervous System
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immune sorbent assay
IFA	Indirect Fluorescent Antibody
HA I	Indirect Haemagglutination Assay
MRI	Magnetic Radiographic Image
T. gondii	*Toxoplasma gondii*
TMC	Tripoli Medical Centre

المستخلص

خلفية :

داء المقوسات هو مرض ينتج عن الإصابة بطفيل التوكسوبلازما *Toxoplasma gondii* ، واحدة من الطفيليات الأكثر شيوعا في العالم. قد يسبب داء المقوسات أعراض شبيهة بأعراض الانفلونزا في بعض الناس، ولكن في معظم الناس لا تتطور الأعراض والعلامات. أما بالنسبة للأطفال الصغار الذي أنجبتهم أمهات مصابات بالمرض وكذلك الأشخاص الذين يعانون من ضعف جهاز المناعة، يمكن لداء المقوسات أن يسبب مضاعفات خطيرة للغاية.

أهداف الدراسة:

لتحديد الانتشار المصلي من عدوى التوكسوبلازما في المرضى الذين يعانون من أورام في طرابلس، ليبيا.

المواد والطرق:

أجريت الدراسة في طرابلس، وشملت 211 مريضا بالسرطان، من الذكور والإناث، تتراوح أعمارهم من 16-80 عاما وقد تم تحليل عينات من دمائهم لغرض الكشف عن وجود الأجسام المضادة لطفيل التوكسوبلازما *T. gondii* IgG and IgM باستخدام تقنية ELISA وكذلك تم تعبئة إستبيان لمعظم المرضى لتوضيح عوامل الإختطار.

نتيجة:

وأظهرت النتائج أن معدل إنتشار مرض التوكسوبلازما في مرضى السرطان كان (45.9٪). أظهرت الفئة العمرية 48-55 أعلى نسبة 54.5٪ والفئة العمرية 16-23 أظهر أدنى معدل. أظهرت مجموعة الإناث اللاتي خضعن للعلاج الكيميائي نسبة ارتفاع بمعدل 49٪ بالمقارنة بالذكور 29٪. ونفس النمط ظهر أيضا في المجموعة غير المعالجة حيث كانت نسبة الإناث 15٪ في حين أن الذكور بنسبة 4٪.

يبدو أن معدل إنتشار مرض التوكسوبلازما في مرضى السرطان مرتفعة وربما تشير هذه النتيجة أن مرض التوكسوبلازما يحتمل أن يزيد من إختطارالاصابة بسرطان الدماغ .

Abstract

Background:

Toxoplasmosis is a disease that results from infection with the *Toxoplasma gondii* parasite, one of the world's most common parasites. Toxoplasmosis may cause flu-like symptoms in some people, but most people affected never develop signs and symptoms. For infants born from infected mothers and for people with weakened immune systems, toxoplasmosis can cause extremely serious complications.

Objective:

To determine the seroprevalence of *T. gondii* infection in patients with tumours in Tripoli, Libya.

Material and Method:

The study was conducted in Tripoli included 211 cancer patients, males and females, their ages range from (16-80 years) their blood samples were analysed for the presence of anti *T. gondii* IgG and IgM antibodies using ELISA and a questionnaire was also filled in for most patients to elucidate risk factors.

Result:

Results showed that the seropositivity rate of anti *T. gondii* antibodies in cancer patients was (45.9%). Age group 48-55 showed the highest (54.5 %) and age group 16-23 showed the lowest rate. The treated female group showed higher rate 49% than the males 29 %. The pattern was also shown in the untreated group where females rate was 15% while males 4%.

It appears that the seroprevalence in cancer patients is high and this result may indicate that *T. gondii* potentially increases the risk of brain cancers in humans.

Chapter 1

INTRODUCTION

1. Introduction

1.1. Definition

Toxoplasmosis is a parasitic disease caused by the protozoan *Toxoplasma gondii* (Ryan and Ray, 2004). The parasite infects most genera of warm-blooded animals, including humans, but the primary host is the felid (cat) family. Animals are infected by eating infected meat, by ingestion of feces of a cat that has itself recently been infected, or by transmission from mother to fetus. Cats are the primary source of infection to human hosts, although contact with raw meat, especially pork, is a more significant source of human infections in some countries (Torda, 2001).

The protozoan was first discovered by Nicolle & Manceaux, who in 1908 isolated it from the African rodent *Ctenodactylus gondii*, then in 1909 differentiated from *Leishmania* and named it *Toxoplasma gondii*. The first recorded congenital case was not until 1923, and the first adult case not until 1940 (Sukthana, 2006). In 1948 , a serological dye test was created by Sabin & Feldman, which is now the standard basis for diagnostic tests, Study Toxoplasma Serology Laboratory (Edwrrd, 1999; Garcia, 2007).

There are 3 major genotypes (type I, type II, and type III) of *T. gondii*. These genotypes differ in their pathogenicity and prevalence in people. In Europe and the United States, type II genotype is responsible for most cases of congenital toxoplasmosis (Lindsay and Dubey, 2011).

T. gondii infects a large proportion of the world's population (perhaps one third) but uncommonly causes clinically significant disease . However, certain individuals are at high risk for severe or life-threatening toxoplasmosis including fetuses, newborns, and immunologically impaired patients (Monotoya and Liesenfeld, 2004).

1.2. Epidemiology

T. gondii has been found worldwide in many species, including carnivorous and herbivorous mammals and birds. It has also been found in every population group of humans investigated. However, the definitive host was shown to be cats, which have been

associated with transmission of the parasite in every population investigated. In the United States, most infection occurs through ingestion of contaminated meat, especially pork and lamb. One survey of pigs in the USA found a seroprevalence of 42% in breeder pigs and 23% in market pigs. Another study in the 1960s found *T. gondii* in 32% of pork chops and 4% of lamb chops in grocery stores. In humans the seroprevalence is 22.5% at the national level, according a study of 17,658 people from 1988 to 1994. Because there is extra concern of congenital infection in mothers, additional attention given to the prevalence in women of child-bearing age. The seroprevalence in the group of 15 to 44 year old women was reported to be 15% (Mandell *et al.*, 2005).

1.3. The Life cycle

The life cycle of *T. gondii* was first described in 1970, when it was discovered that the definitive hosts are members of the family Felidae, including domestic cats. Various warm-blooded animals, birds and human serve as intermediate hosts (Dubey and Beatty,1988; Garcia *et al*., 2007 ; Jayaram, 2007).

The life cycle of *T. gondii* has two phases. The sexual part of the life cycle (coccidian like) takes place only in cats, both domestic and wild (family *Felidae*), (Boothroyd, 2009) which makes cats the parasite's primary host. The second phase, the asexual part of the life cycle, can take place in other warm-blooded animals, including cats, mice, humans, and birds. The hosts in which asexual reproduction takes place is called the intermediate host. Rodents are the typical intermediate host (Boothroyd, 2009).

The life cycle of *Toxoplasma gondii* begins as an unsporulated oocyst, which is a non-infective egg cell. These cells sporulate and make sporozoites in the environment to become infective. These are found in the feces of infected cats (Noble and Noble, 1976).

Water, surrounding soil, and food can become contaminated with these infective cells. When these are ingested by a mouse, or another intermediate host, the sporozoites invade almost every kind of tissue in the host's body. They then begin to multiply asexually by endodyogeny. At this point the sporozoites are called tachyzoites. They

multiply very quickly forming pseudocysts inside host cells, which burst open to release more tachyzoites. After the first few days of infection, the tachyzoites are called bradyzoites. They multiply more slowly and stop bursting out of pseudocysts. They form shelled cysts to hide in the host's tissues until the second and final host, the cat, eats the intermediate host (Sleigh, 1973; Noble and Noble, 1976).

Inside the cat, the cysts go through gametogony formation of zygotes, then oocysts, then sporonts, then sporoblasts, and finally sporozoites to complete the sexual life cycle (Noble and Noble, 1976; Hausmann and Hulsmann, 1996).

T. gondii also infects humans as an intermediate host, causing toxoplasmosis. This can happen if a human ingests undercooked or contaminated meat. A human can also be infected by coming in close contact with the contents of cat litter boxes, which is one reason the percentage of infection among humans is so high (Dubey *et al.*, 1973).

Once inside a human, *T. gondii* undergoes the same rapid multiplication in which flu like symptoms might be happen, then settles into cyst formation and hiding, however it is unlikely that the human will ever be eaten by the secondary host, the cat. Macrophages are effective in providing immunity against *T. gondii* (Haussmann and Hulsmann, 1996).

If women are infected for the first time after pregnancy there is a chance that their infants will be infected as well. This can lead to birth defects or early abortion (CDC, 2008).

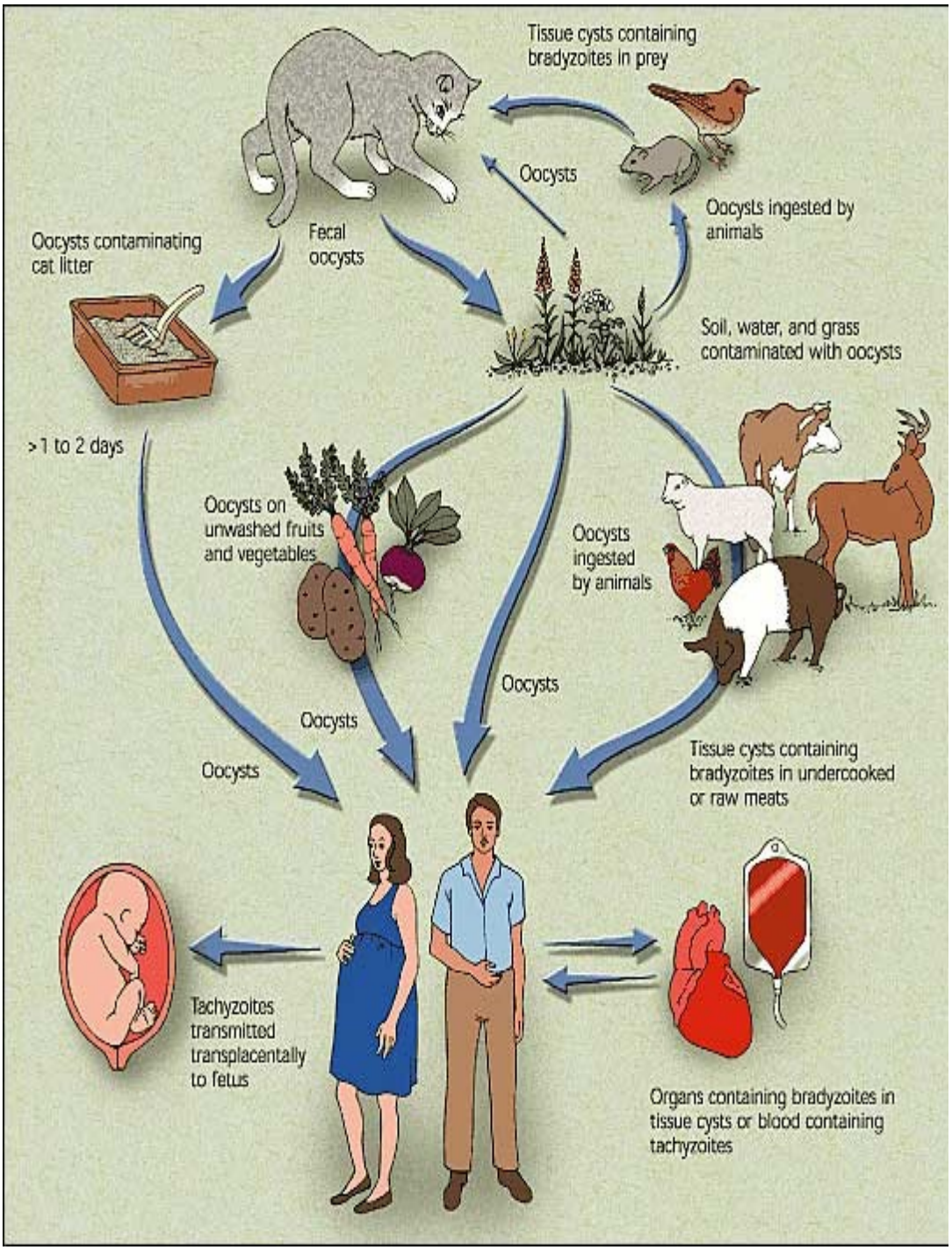


Figure 1. Life cycle and pathways for *T. gondii* infection (Lynfild *et al*., 1997).

1.4. Transmission

Transmission may occur through the following:

- Ingestion of raw or partly cooked meat, especially pork, lamb, or venison containing Toxoplasma cysts. Infection prevalence in countries where undercooked meat is traditionally eaten has been related to this transmission method. Tissue cysts may also be ingested during hand-to-mouth contact after handling undercooked meat, or from using knives, utensils, or cutting boards contaminated by raw meat (CDC, 2004).
- Ingestion of contaminated cat feces. This can occur through hand-to-mouth contact following gardening, cleaning a cat's litter box, contact with children's sandpits, or touching a leech, and can survive in the environment for over a year, Content North Carolina department of agriculture and consumer services .

  Cats excrete the pathogen in their feces for a number of weeks after contracting the disease, generally by eating an infected rodent. Even then, cat faeces are not generally contagious for the first day or two after excretion, after which the cyst 'ripens' and becomes potentially pathogenic (CDC, 2011).
- Congenital transmission to the fetus via the placenta during acute stage of infection (tachyzoites) by blood transfusion or laboratory accidents (Edward *et al*., 1999 ,Jones *et al*., 2003; and Hill, 2005). Tachyzoites (Jayaram, 2007), organ transplantation (bradyzoites in tissue cysts) (Joyner, 1982, Edward *et al*., 1999; Jayaram, 2007).

1.5. Symptoms and signs

In an adult human, the incubation period for *T. gondii* infection ranges from 10 to 23 days after the ingestion of undercooked meat and it is from 5 to 20 days in case of oocyst ingestion from cat faeces (Jones *et al*., 2003).

The clinical manifestations of toxoplasmosis vary greatly, producing a range of nonspecific symptoms. In immunocompetent patients, infection is usually asymptomatic

or very mild. Common symptoms include cervical lymphadenopathy and other symptoms of generalized infection. These include fever, malaise, night sweats, myalgia, sore throat and maculopapular rash (Jayaram, 2007).

The clinical course in immunocompromised patients can be much more severe. These infections usually stem from reactivated latent infection, rather than newly acquired infection (Garcia *et al*., 2007). Important symptoms include encephalitis, myocarditis and pneumonitis; with death being almost certain if the disease is left untreated. In AIDS patients, toxoplasma encephalitis is especially frequent, occurring in 10-50% of seropositive patients with CD4 T-cell counts less than 100/μl (Jayaram, 2007).

Toxoplasmosis in congenitally infected newborns can present in a nonspecific manner with a wide range of symptoms, some of which can also be quite severe. These include chorioretinitis, blindness, epilepsy, mental retardation, anemia, jaundice, encephalitis, pneumonitis and others. Complete recovery is very rare (Mandell *et al*., 2005).

1.6. Diagnosis

Clinical signs of toxoplasmosis are non-specific and cannot be depended upon for a definite diagnosis. *T. gondii* diagnosis is made by serological, biological or histological methods, or by some combination of them. Lab diagnosis is based primarily on antibody detection by using various serologic procedures which are highly specific and sensitive tests (Dubey and Beattie, 1988; Gillespie and Hawkey, 1994; Burton *et al*., 2005; Garcia, 2007).

The diagnosis of toxoplasmosis can be done using a variety of methods. The difficulty lies in determining whether the infection is acute or chronic. Acute infection can best be verified by isolating *T. gondii* or *T. gondii* DNA from the patient's blood or finding tachyzoites in tissue or bodily fluids. Congenital infection of fetuses can be identified by the presence of cysts in the placenta or fetus (Mandell *et al*., 2005).

The isolation of *T. gondii* tissue cysts is not sufficient to determine whether the infection is still active or has entered the latent phase. To differentiate the two, patients are subjected to several serological exams, the specific combination of which depends on clinical category of the patient. These exams include the Sabin-Feldman dye test, which tests for IgG antibodies; ELISAs targeted at IgM, IgA and IgE; differential agglutination tests and IgG avidity tests (Dubey and Beattie 1988; Gillespie and Hawkey, 1994; Burton *et al.,* 2005).

Of particular interest is determining acute infection in pregnant women, due to the risk of congenital toxoplasmosis. This is complicated by the fact that many women have existing IgG and IgM antibodies to *T. gondii* from infection in the past. There are effective diagnostic techniques that monitor changes in the mother's antibody expression over time, but quick diagnosis is greatly preferred because fetuses often rapidly become infected (Mandell *et al*., 2005).

Driven by this need, Cynthia Press, Jose Montoya and Jack Remington at the Palo Alto Medical Foundation identified a series of tests that are effective at determining very early infection from a single sample of maternal serum. This is based on the finding that VIDAS IgG avidity tests usually fail during early infection because IgG titers are too low. The recommended diagnostic procedure consists of looking for positive results from the Sabin-Feldman dye test and an IgM ELISA, in conjunction with a negative VIDAS IgG avidity test. This was determined to be an effective set of criteria for diagnosing early acute infection, which can then be followed up with early treatment to minimize the risk of congenital infection (Mandell *et al* ., 2005).

1.7. Serology

The most commonly used serologic tests detect the presence of anti -*T gondii* IgG and IgM, IgG antibodies can be detected with the Sabin-Feldman dye test (considered the gold standard), indirect fluorescent antibody (IFA), agglutination, or enzyme-linked immunosorbent assay (ELISA 61). IgG titers peak within 1-2 months after infection but

remain elevated for life .Tests that detect IgM antibodies include double sandwich ELISA, IFA, and immunosorbent agglutination assay (ISAGA 61). These tests can be helpful because the absence of anti -*T. gondii* IgM virtually excludes recent infection in immunocompetent patients .Although IgM antibodies typically disappear a few weeks or months after infection, they can remain elevated for more than 1 year. Thus, the presence of anti -*T gondii* IgM antibodies does not necessarily indicate that the infection was acquired recently .This issue is important in the evaluation of pregnant women because congenital transmission of *T gondii* in immunocompetent women occurs almost exclusively when infection is acquired during gestation .Differential agglutination compares titers obtained with methanol-fixed tachyzoites (AC antigen) and formalin-fixed tachyzoites HS antigen) .(The AC/HS ratio is useful to distinguish acute from chronic infection (Mandell, 2000).

1.8. **Treatment**

Treatment of toxoplasmosis in immunocompetent patients is usually unnecessary. In immunocompromised patients, the recommended treatment is a combination of pyrimethamine given at 25-100 mg daily and trisulfapyrimidines given at 2-6 g daily, both for a month. This drug combination inhibits dihydrofolate reductase in *T. gondii*, preventing synthesis of DNA and protein. Folinic acid can also be administered to reduce bone marrow depression caused by the pyrimethamine. Clindamycin has been found to be effective at treating toxoplasma encephalitis in AIDS patients (John *et al*., 2006).

Infants with congenital toxoplasmosis and acute adult cases as immunosuppressed patients are generally treated by Pyrimethamine (Daraprim) and Sulfonamides (Dubey and Beattie,1988; Remington *et al.*, 2001; Petersen *et al.,* 2003).

In acutely infected pregnant women, the recommended treatment includes spiramycin if the foetus has not yet acquired toxoplasmosis. Spiramycin is an antibiotic that localizes to the placenta and has been shown to reduce placental infection by 60%. It does have some teratogenic effects, which must be weighed against the risk of congenital

infection. If the fetus is infected, the aforementioned drug combination is administered instead of spiramycin (John *et al*., 2006).

Women who have acquired *T. gondii* infection during pregnancy are treated with spiramycin (Rovamycine) to prevent transmission of *T. gondii* from the placenta to the fetus (Singh, 2003; Garcia *et al*., 2007).

1.9. Prevention and control

Prevention of primary infection is currently best achieved through health recommendations (Mandell *et al*., 2005; John *et al*., 2006).

1-Preventive procedures related to cats:

- Cat owners warned to avoid directly handling litter trays or soil that may be contaminated with cat (Mandell *et al*., 2005; John *et al*., 2006).
- Cats should never be fed uncooked meat (Ito *et al*., 1975; Dubey, 1986; Edward *et al.* ,1999).
- Clean your cat's litter box. Wear gloves and a face mask to change the litter (Foulon, *et al*., 1994).

2- Preventive procedures related with Food:

- Do not eat undercooked meat.
- Meat should be thoroughly Cooked to at least 145° F (63° C).
- Freeze meat for several days at sub-zero (0° F) temperatures before cooking to greatly reduce chance of infection (Mandell *et al*., 2005), (John *et al*., 2006; Montoya, 2011).
- Peel or wash fruits and vegetables thoroughly before eating Avoid drinking untreated drinking water. (Dubey, 1988; Dubey and Beattie,1988; John *et al*., 2006).

3-Preventive procedures related with pregnant women and those with weakened immune systems should take the following precautions:

- Do not clean cat litter boxes.

- Do not touch anything that could be contaminated by insects exposed to cat faeces (cockroaches, flies, etc. Pregnant women and those with HIV should be screened for toxoplasmosis .A blood test can be done.
- Do not touch anything that may contain cat faeces, (Dubey, 1988; Dubey and Beattie, 1988).

Chapter 2

- **Literature review**
- **Objectives of the study**

2.1. Literature review

Austria implemented a toxoplasmosis screening program in 1975 .Nearly all women who become pregnant are serologically screened early in pregnancy and, if found to be negative initially, are tested again during the second and third trimesters. Women with Toxoplasma infections are treated as soon as infection is detected .Although seropositivity rates among pregnant Austrian women have declined from approximately 50% during the late 1970s to 36.7% during the early 1990s, the incidence of congenital Toxoplasma infection has declined even more, from 50-70 cases per 10,000 births before the program to 1 per 10,000 births during the early 1990s (Spock *et al.*, 1992).

During June 1992 and August 1996, researchers in Denmark conducted a newborn screening study for toxoplasmosis .The primary goal of this study was to determine the feasibility of screening newborn infants for congenital toxoplasmosis in an area with low prevalence; in Denmark, the seroprevalence of antibodies to Toxoplasma among women during this study was 28% (Lebech *et al*., 1999; Lynfield *et al*.,1999). Approximately 90,000 infants were screened for Toxoplasma- specific IgG antibodies 5-10 days after birth .Infants born to mothers who seroconverted during pregnancy were subsequently examined physically and serologically for 1 year; for those with confirmed congenital infections, treatment was initiated with courses of pyrimethamine and sulfadiazine, alternating with spiramycin. During 1996, serum levels of Toxoplasma-specific IgM antibodies were also determined. The IgM test conducted within 10 days of birth resulted in a false-positive rate of 0.2 per 1,000 with no false-negatives .Results from this study indicated that a newborn screening program using a Toxoplasma- specific IgM antibody test exclusively could identify approximately 75% of infections in infants born to untreated mothers. In addition, the low rates of false-positives and false-negatives suggested this method would be feasible in large-scale newborn screening programs in areas with low seroprevalence rates of toxoplasmosis (Lebech *et al*., 1999; Lynfield *et al.*, 1999).

To determine *Toxoplasma gondii* antibodies in cancer patients, 267 cancer patients were studied using ELISA and higher positivity rates of *T. gondii* IgG were detected than the control. The positivity rates of *T. gondii* IgG in nasopharyngeal carcinoma, rectal cancer groups were significantly higher than the other cancer groups, but the differences in IgM positivity rates were not significant, demonstrating that there is a likely association between *T. gondii* infection and some kinds of cancer, especially nasopharyngeal carcinoma and rectal cancer (Yuan *et al.,* 2007).

In 2007, researchers in Iran conducted study to determination of Antibodies (IgG, IgM) against *Toxoplasma gondii* in Patients with Cancer. This study was carried out on 252 malignant patients and 252 healthy normal subjects. Serum samples of patients were examined for IgG and IgM antibodies by ELISA technique, The results of this study revealed the presence of toxoplasma antibodies in 114 (45.2%) cases of patients who were positive for Toxoplasma IgG antibodies, and 26 (10.3%) cases were confirmed to be positive for Toxoplasma IgM antibodies and also 17 (6.7%) of cases had both IgG and IgM antibodies against *Toxoplasma gondii*. In control group 92 (36.5%) cases and 15 (6%) cases revealed seropositive for IgG and IgM antibodies, respectively. There were no significant differences between sex, close contact with cat, living region, chemotherapy, and seropositivity rate of toxoplasmosis inpatients. Comparing the age groups, the highest seropositive rate showed in the age of 51 years or higher, and their rates had tendency to increase with age in both groups. No seropositivity significant relationship was found between patients and control group (Ghasemian *et al.,* 2007).

A recent work conducted in France showed that mortality rates due to brain cancer correlated positively with the local sero-prevalence of Toxoplasma , particularly in the people who are 55 years of age or olderm (Vittecoq *et al.,* 2012).

Frederic *at al.*,(2011) predicted in their studies that *T. gondii* could increase the risk of brain cancer because it is a long-lived parasite that encysts in the brain, where it provokes inflammation and inhibits apoptosis. They are used a medical geography approach based on the national incidence of brain cancers and seroprevalence of *T. gondii*. They are corrected reports of incidence for national gross domestic product

because wealth probably increases the ability to detect cancer. They are also included gender, cell phone use and latitude as variables in our initial models. Prevalence of *T. gondii* explained 19 per cent of the residual variance in brain cancer incidence after controlling for the positive effects of gross domestic product and latitude among nations. Infection with *T. gondii* was associated with a 1.8-fold increase in the risk of brain cancers across the range of *T. gondii* prevalence in our dataset (4-67%). These results, though correlational, suggest that *T. gondii* should be investigated further as a possible oncogenic pathogen of human (Frederic, 2011).

Up to a third of the world's human population is estimated to carry a Toxoplasma infection (Jones *et al.*, 2007). The Centre for Disease Control and Prevention noted that overall seroprevalence in the United States as determined with specimens collected by the National Health and Nutritional Examination Survey (NHANES) between 1999 and 2004 was found to be 10.8%, with seroprevalence among women of childbearing age (15 to 44 years) 11%. Another study placed seroprevalence in the U.S. at 22.5%. The same study claimed a seroprevalence of 75% in El Salvador (Jones *et al*., 2007).

A growing body of literature suggests that exposure to the neurotropic parasite *Toxoplasma gondii* (*T. gondii*) is associated with increased risk of mental disorders, particularly schizophrenia (Markovitz, 2015).

In 2014, Zhongguo found that IgG positive rate of *T. gondii* is very high in Pu'er City, and therefore, the health education for toxoplasmosis control should be strengthened

The parasites are able to induce direct modifications in the infected cells, for example by altering dopamine metabolism, by functionally silencing neurons as well as by hindering apoptosis. Moreover, indirect effects of the peripheral immune system and alterations of the immune status of the CNS, observed during chronic infection, might also contribute to changes in neuronal connectivity and synaptic plasticity (Dunay *et al.,* 2015).

2.2. Epidemiology of toxoplasmosis in Libya

In 1980 serological survey for toxoplasmosis in Tripoli, Libya showed that Toxoplasma antibodies were found at a titre of 1:16 or above in 51.6% of 2000 adult males, in 43.4% of 300 adult females, and in 43.7% of 1980 school children (7-18 years of age) from the area of Tripoli (Libya).However, 69.4% of a group of 1921 female patients with a history of abortion and clinically suspected of being infected with *T. gondii* had Toxoplasma antibodies, and 18.6% of them exhibited serological evidence of recent infection 4-fold or greater increase in antibody titre over 2-3 weeks (Khadre *et al.,* 1987).

In 1991 a study conducted in Benghazi screened 369 pregnant women with manifestations suggesting *T. gondii* infection and used IHAT in detection analysis the result indicated that 47.4% of them were has *T. gondii* antibody. The high rate of positivity was among the old age group (63.3%). and in this study recommends for necessary examined before the marriage for anti *T. gondii* antibodies. Also married women must be examined, if not examined before, to follow any rise in the titre (Kassem and Morsy, 1991).

A more intensive study investigating 1567 individual was also carried out by Gashout et al (1992) in Tripoli central Hospital by using IHA test and showed an overall incidence of 38.48% the seropositivity of sera obtained from female was 41.13% out of 1388 samples. The results of seroconversion rates in males was 17.87% out of 179 sera tested.

In 2003 a study by Magrhi on investigation of the epidemiological features and pregnancy outcome of the pregnant women ended with miscarriage and were seropositive for toxoplasmosis in the Tripoli Medical Centre (TMC) and AL-Jala Maternity Hospital (AMH). The total number of samples was 1000, in which 569 patients from (TMC) and 431 from (AMH). Latex haemagglutination test (toxo latex) was used for antigen antibody reaction, and for an ELISA. For all patients whom are seropositive are test is being done in three weeks to assess the serological changes and to assess the progress of

the disease. For the patients who are pregnant and IgM was positive a follow-up with a serial ultrasound scanning for the foetus was done and Histopathological examination of the placenta after delivery was performed. The seropositive patients were 31.29% of the patients in both hospitals and it was at the age group 31-35.the seropositive patients at TMC were 235 out of 569 patients (41.30%) while at AMH the number was 206 out of 431 (47.80%), for both hospitals the number was 441 out of the total of 1000 (44.10%). The results reveal some interesting factors contributing to the spread of the disease drinking of water from wells which could be contaminated Conducted a study by In (2008) Abu Setta and Yamani approximately 474 non pregnant women in Tripoli, Libya were investigated for *Toxoplasma gondii* by ELISA. The seroprevalence rate of toxoplasmosis (18.14) in all participants also other study by Alkhunfas (2008) in Al -Jala Maternity Hospital Tripoli ,Libya found that seroprevalence of *T. gondii* IgG antibodies was 39.45% (130 mothers of 330) It was at its highest (44%) for newborn babies of mothers aged between 30-35 years.

Gashout *et al*. (2008) tested 692serum samples for the presence of antibodies for toxoplasmosis and rubella using ELISA techniques. The investigations were carried out in the laboratory of Tripoli Central Hospital from January 2002 to December 2007. Testing results showed serological evidence of previous 4.3% for rubella with infection in 17.6% of tested women for toxoplasmosis and only. co-existence of both infections in only 1.8% of women also other study by Azbida (2009) in Alrazi Neuropsychiatry Hospital, Tripoli, Libya by using ELISA and Latex found that seroprevalence of ant *T. gondii* IgG antibodies by Latex was 61.7% in patients and 46.7% in control samples, while by ELISA was 50.3% in patients 33.0% in control samples.

A study done by (EL-Khgkhag, 2010) using ELISA 406 pregnant women aged 18-45 years attending the AL-Wahda Hospital in Derna city were tested for the prevalence of anti-*T. gondii* antibodies. The seroprevalence of anti-Toxoplasma antibodies in pregnant women in Derna city was found to be 40%, among which was showing a titer of 31,5% and 8.4% for IgG and IgM respectively. The highest IgG and IgM seroprevalence were among pregnant women aged 36 to 45 years (74.3%).

A study by (Mousa *et al.*, 2011) in Benghazi, Libya, found that seroprevalence of *T. gondii* IgG and IgM antibodies were found in (44.8%), (8.4%) of 143 pregnant by ELISA respectively.

2.3. Objectives of the study

- **Main objective**

To determine the seroprevalence of *T. gondii* infection in patients with tumours in Tripoli, Libya.

- **Secondary objective**

To determine the relationship between treated patients and the results of *T. gondii* by ELISA.

Chapter 3

Materials & methods

3. Materials & methods

3.1. Samples of the study

This study was conducted on 211 samples collected from patients, males 72 and females number 139 with tumors in Tripoli Medical Centre (TMC) which is considered to be the biggest Hospital in Tripoli, Libya.

Questionnaire form was designed to assess some of the main risk factors which may influence the prevalence of toxoplasmosis infection including, Socio-demographic data (name, age, sex, residence place, educational level), and behavioural data (blood transfusion, eating habits, and contact with animals especially cats and dogs).

3.2. Collection of blood samples

Venous blood sample of (5ml) was collected from each patient and then transferred to the laboratory, centrifuged at 1000 r.p.m. for 5 mins for separation of the serum then stored at -20℃ until the analysis

3.3. Laboratory assay

The serum obtained was screened for ELISA IgG and IgM using kits of (Biocheck, Inc.) for serological identification of anti-*Toxoplasma gondii.*

3.3.1 Reagents

a) Materials provided with the Kit

- Microtiter Wells: Toxoplasma antigen-coated wells (12x8 wells).
- Enzyme Conjugate Reagent (red colour): Red cap 1 vial (12 ml).
- Sample Diluent (green color): 1 bottle (22 ml).
- Negative Calibrator: 0 IU/ml.Natural cap (150 Ul /vial).
- Cut-off Calibrator: 32 IU/ml .Yellow cap (150 Ul/vial).
- Positive Calibrato: 100 IU/ml .Red cap (150 Ul/vial).
- Positive Calibrato: 300 IU/ml.Green cap (150 Ul/vial).

- Negative Control: Range stated on label .Blue cap (150 Ul/vial).
- Positive Control: Range stated on label. Purple cap (150 Ul/vial).
- Wash Buffer Concentrate (20 x): 1 bottle (50 ml).
- TMB Reagent (One-Step):1 vial (11 ml).
- Stop Solution: 1N HCL. Natural cap. 1 vial (11ml).g

b) Materials needed but not provided with the Kit:

- Micropipettes and disposable tips.
- 37 ℃ incubator.
- Mechanical washer.
- Spectrophotometer.

3.3.2. Reagent Preparation

- All reagents were allowed to reach room temperature (18-25℃) before use.
- Dilute 1 volume of Wash Buffer (20 x) with 19 volumes of distilled water.
- Mix well be (150 Ul/vial) free use.

3.3.3. Assay procedure

1. The desired number of coated wells were placed into the holder
2. Were prepared 1: dilution of test samples, negative control; positive control and calibrators by adding 5 *µl*. of the sample to 200 *µl*. of sample Diluent. Mix well
3. Dispense 100 *µl*. diluted sera, and controls into the appropriate wells. For the reagent blank, dispense 100 *µl*. Sample Diluent in 1A well position. Tap the holder to remove air bubbles form the liquid and mix will.
4. Incubate at 37º C for 30 minutes.
5. At the end of incubation period, remove liquid form all wells. Rinse and flick the microtiter wells 4 times in the diluted Wash Buffer (1x) and then one with distilled water
6. Dispense 100 *µl*. of Enzyme Conjugate to each well. Mix gently for 10 seconds.
7. Incubate at 37º C for 30 minutes.

8. Remove Enzyme Conjugate form all wells. Rinse and Flick the microtiter wells 4 times with diluted Wash Buffer (1x) and then one time with distilled water.
9. Dispense 100 *µl*. of TMB Reagent into each wells. Mix gently for 10 seconds.
10. Incubate at 37º C for 15 minutes.
11. Add 100 *µl*. of stop Solution (1 N HCL) to stop reaction.
12. Mix gently for 30 seconds. It is important to make sure that all the blue color changes to yellow colour completely.
13. Read O.D. at 450 nm within 15 minutes with a micro well reader.

3.4. Calculation of results

1) Results were calculated by the mean of duplicate cut-off (32 IU/ml) calibrator value xc.
2) Calculate the mean of duplicate positive control (xp), negative control (xn) and patent samples (xs).
3) Calculate the Toxoplasma IgG Index of each determination by dividing the mean values of each sample by calibrator mean value, xc.

3.5. Interpretation

- Negative: Toxo G Index less 0.91 indicates absence of prior exposure to *Toxoplasma* (32 IU/ml).
- Equivocal: Toxo G Index between 0.91 -0.99 is equivocal. Sample should be retested.
- Positive: Toxo G Index of 1.00 or greater or WHO IU/ml value greater than 32 IU/m is seropositive. It indicates prior exposure to the *Toxoplasma*.

3.6. Statistical analysis

The statistical package for social science (prism5) was used for statistical analysis of data to optimise results of this study. The significant comparisons were considered when $P \leq 0.05$

المركز الوطنى لمكافحة الامراض

نموذج استبيان بخصوص بحث معدل انتشار مرض المقوسة القندية في الاطفال المشوهين خلقيا

لسنة 2013

Questionnaire Form

الاســم ☐ **العمر** ☐

رقم العينة ☐ **الاقامة** ☐

الجنس:-ذكر ☐ **انثي** ☐

المستوي التعليمي **روضة** ☐ **ابتدائي** ☐ **إعدادي** ☐

تربية الحيوانات :- **نعم** ☐ **لا** ☐

نوع الحيوانات :- **قطط** ☐ **كلاب** ☐

اتصال الام مع الحيوانات **القطط** ☐ **الكلاب** ☐

هل تعرضت الام لنقل دم **نعم** ☐ **لا** ☐

نتيجة الاختبار(IgG) ELISA **سالب** ☐ **موجب** ☐

ELISA(IgM) **سالب** ☐ **موجب** ☐

Chapter 4

Results

4. Results

The results of the serological analysis revealed that 97 (45.9%) were seropositive for *T. gondii* by ELISA IgG. See details in (Table 1)

Table 1. Relationship between total results of *T. gondii* by ELISA IgG.

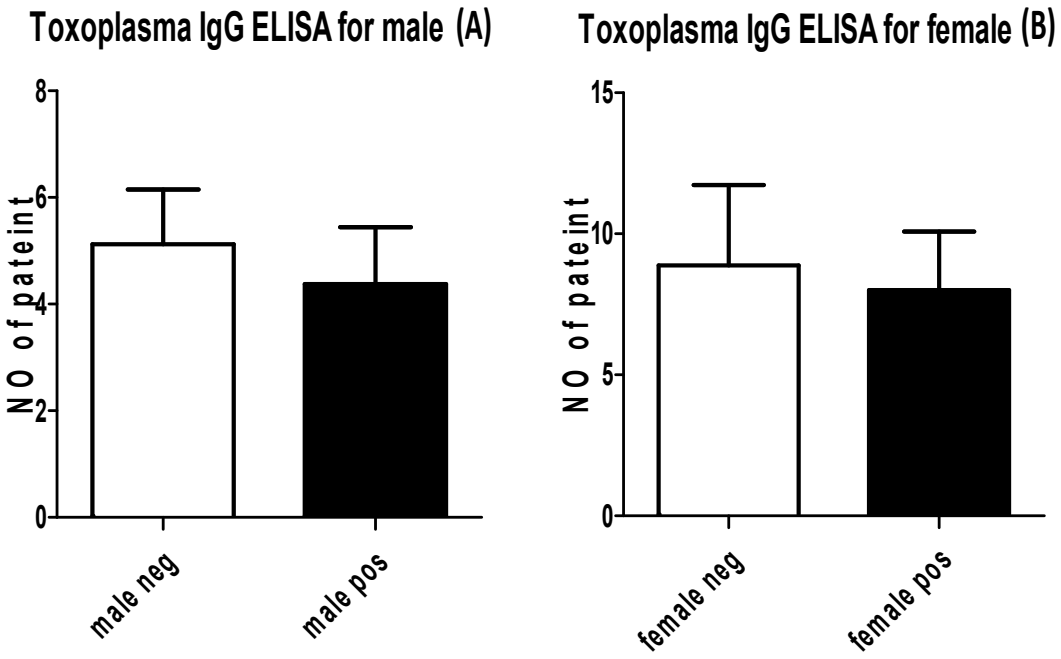
Cases	Total	ELISA			
		Positive		Negative	
	Number	Number	%	Number	%
Patients	211	97	45.9	114	54.1

4.1. Relationship between age group and results of *T. gondii* by ELISA

In age group comparisons, the highest seropositive rate showed in the age of 72 > years where the highest percentage reached 61.5%, while the lowest rate in the age group (16-23), where the percentage reached (25 %) (Table 2) ;statistically significant difference ($p < 0.05$).

Table 2. Relationship between age group and results of *T. gondii* by ELISA IgG:

Age Group	ELISA			
	Positive		Negative	
	Number	%	Number	%
16-23	2	25	6	75
24-31	5	41.7	7	58.3
32-39	10	43.5	13	56.5
40-47	21	40.4	31	95.6
48-55	24	54.6	20	45.4
56-63	16	43.2	21	56.8
64-71	11	50	11	50
72 >	8	61.5	5	38.5
Total	97	386,9	114	476.1



Mean ± SEM of column A	5.125 ± 1.025 N=8
Mean ± SEM of column B	4.375 ± 1.068 N=8

Mean ± SEM of column A	8.875 ± 2.850 N=8
Mean ± SEM of column B	8.000 ± 2.079 N=8

Figure (1) showed ELISA Toxoplasma results in cancer patient for males and females. In (A) and (B) No statistical significant difference, the p value was 0.620 and 0.803 by using t test `` Prism5 software. Statistically significant difference $p < 0.05$.

4.2. Distribution of *T. gondii*, results by ELISA IgG between males and females:

In comparison between males and females in the same age group, it was found that females in the age group (16-23) and (72 >) had the lowest infection rate and was highest in the age group (40-47) and in the males recorded the highest proportion in the category of age (48-55) and less in proportion (24-31). No significant difference in both A and C and the p value was 0.38, 0.88 respectively by using Chi Square test.

Table 3. Shows the relationship results between *T. gondii* IgG in both sexes

Age Group	ELISA			
	Male		Female	
	Positive NO	Positive %	Positive No	Positive %
16-23	1	0.5	1	0.5
24-31	0	0	5	2
32-39	2	1	8	4
40-47	4	2	17	8
48-55	9	4	15	7
56-63	6	3	10	5
64-71	4	2	7	3
72 >	7	3	1	0.5
Total	**33**	**16**	**64**	**30**

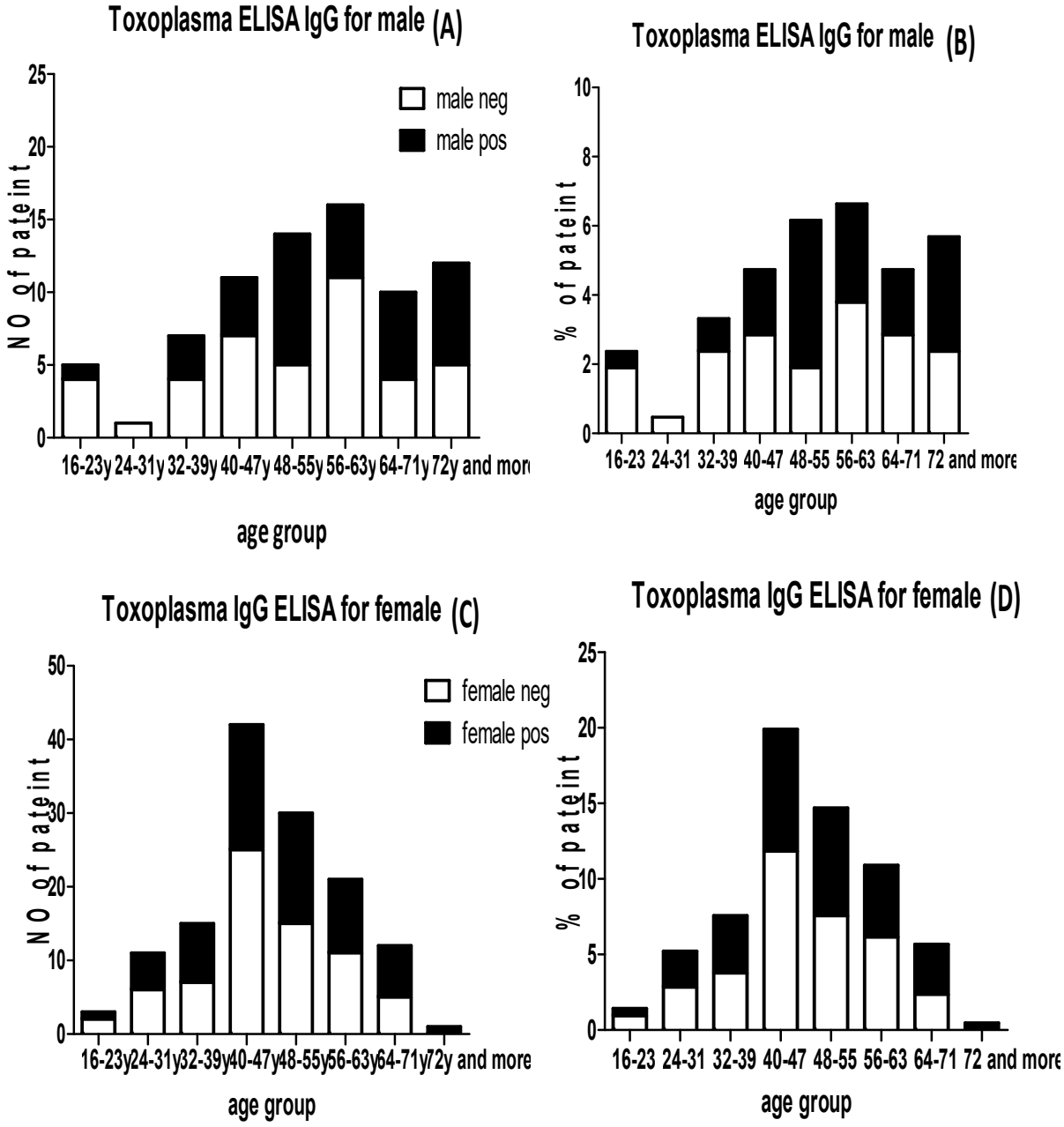


Figure (2), Toxoplasma ELISA results for male and female which was divided to different age groups in both male and female. In A, and C was comparing the number of pateint with age group while in B, D was comparing the percntage of pateint with age group. No significant difference in both A and C and the p value was 0.38, 0.88 respectively by using chi square test, GraphPad Prism5 software.

3.3 .Distribution of Percentage *T. gondii* , results by ELISA IgG cancer patients.

The results of serological analysis revealed that, 97 (45.9%) of patient samples were seropositive for *T. gondii* by ELISA IgG The serological results showed that the positive cases were:- In female (30%) and male(16%)

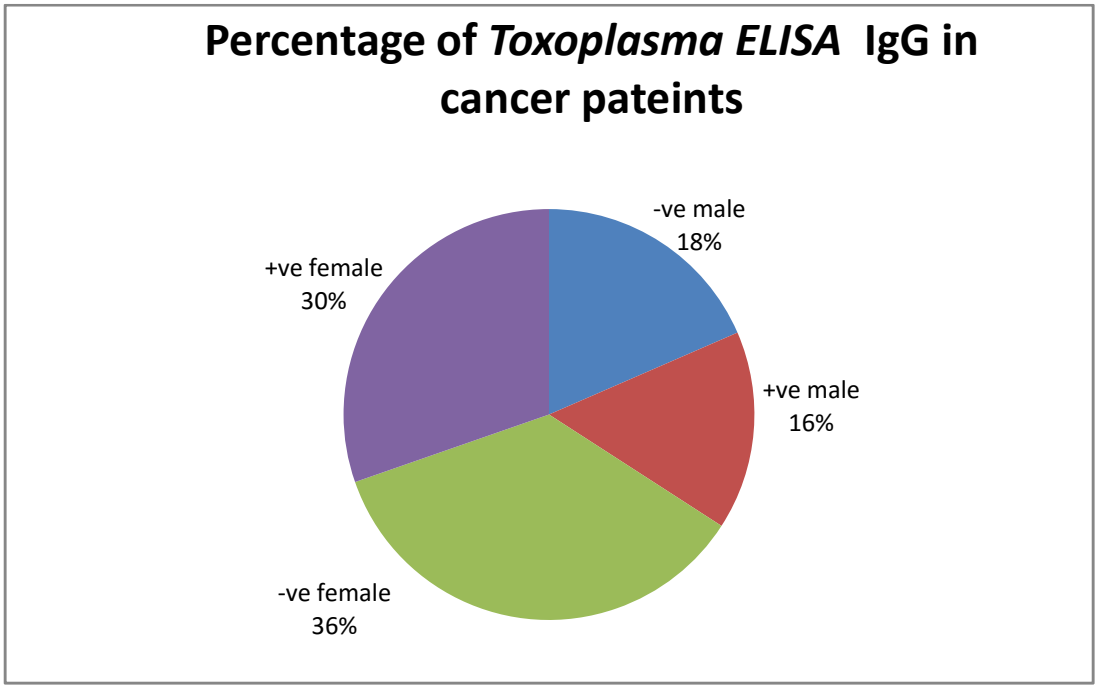


Figure 3. Percentage of *Toxoplasma* IgG in ELISA results for cancer patients.

4.4. Relationship between treated (Chemotherapy), and results of *T. gondii* using ELISA

Among the total of 211 samples collected, 58 were from male treated patients and 109 were from female treated patients in which the seroprevalence was 29 (50%) and 49 (44.9%) respectively. See Table 6 and Figure 4 for details.

Table4. Relationship between treated (Chemotherapy) and results of *T. gondii* by ELISA

Gender	Treated	Untreated	Positive IgG, treated		Positive IgG, untreated	
			No	%	NO	%
Male (72)	58	14	29	50	4	28.5
Female (139)	109	30	49	44.9	15	50
Total	**167**	**44**	**78**	**46.7**	**19**	**43.1**

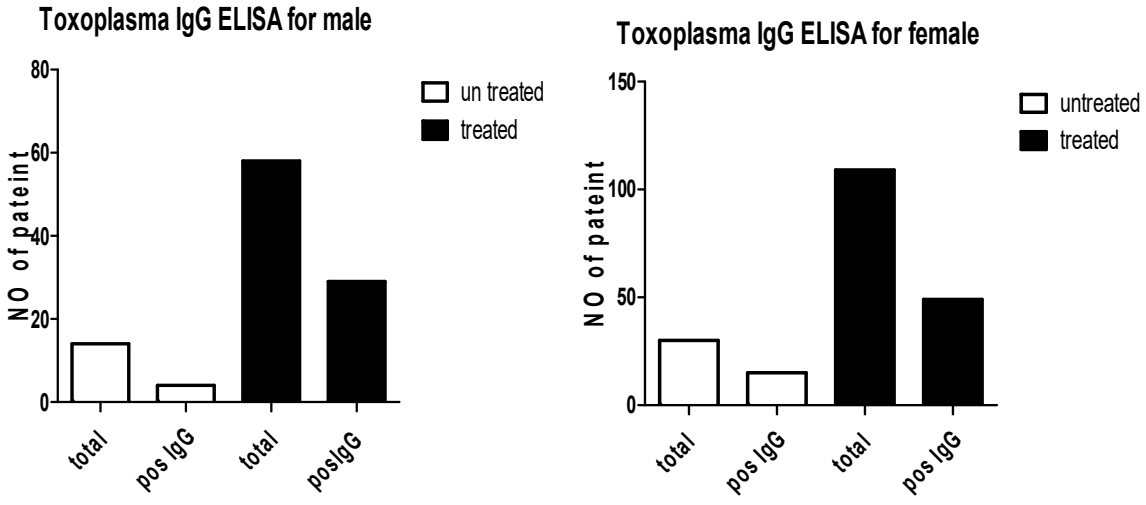


Figure (4), Toxoplasma ELISA results for cancer patient which divided into, un treated with chemotherapy and treated with chemotherapy in both male and female. In both the P value was 0.3 un significant by using one way ANOVA

Table5. Distribution of IgM of *T. gondii* results by ELISA in males and females

Gender	Number	Percentage %
Negative male	71	33.65
Positive male	1	0.47
Negative female	129	61.14
Positive female	10	4.74

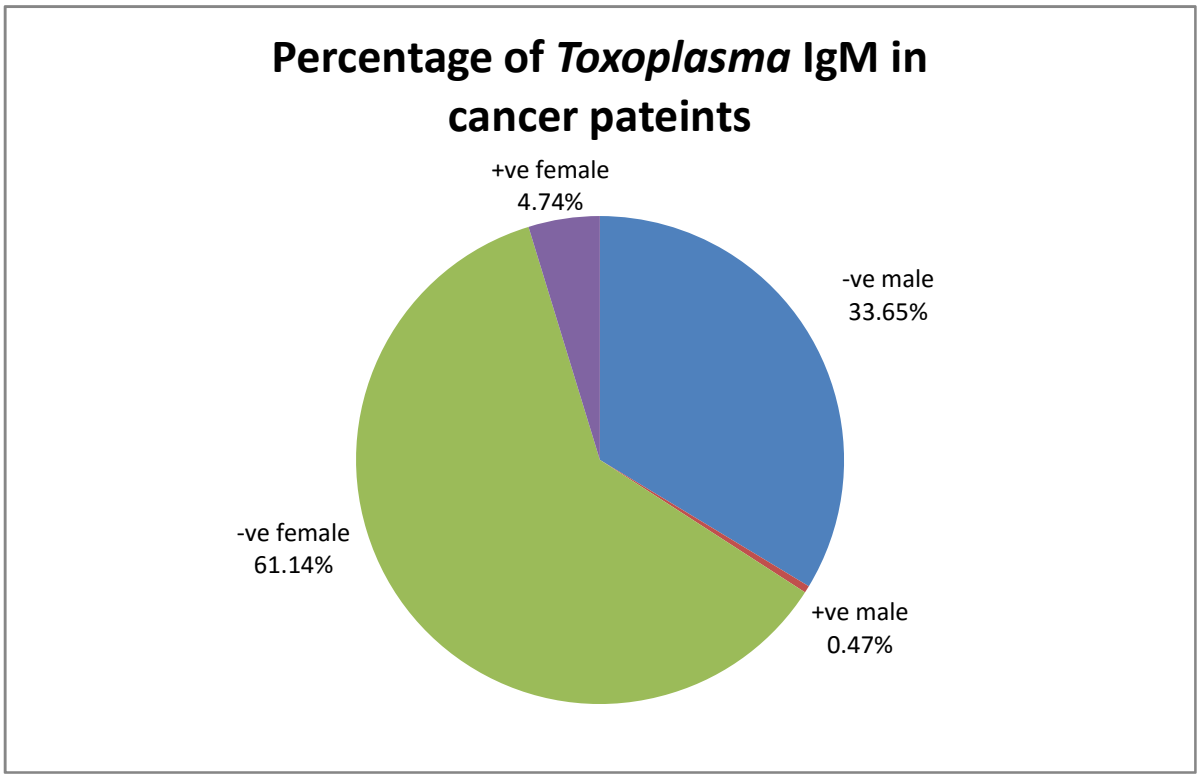


Figure (5) Percentage of T*oxoplasma* IgM by ELISA results for cancer patients.

Table6. Distribution of IgG & IgM *T. gondii* results by ELISA in males and females

Gender	Positive IgG & IgM	Negative IgG & IgM
Male	1	71
Female	6	133

4.4. Determination of antibodies (IgG, IgM) against *Toxoplasma gondii* in patients with Cancer

The study found that the proportion of (IgG) from the highest spectrum (IgM) where 45.9% and 5 % respectively (Table 7, 8)

Table7. Comparison between (IgG, IgM) against *Toxoplasma gondii* in Patients with Cancer

	IgG		IgM	
	Number	(%)	Number	(%)
Positive	97	(45.9)	11	(5.2)
Negative	114	(54.1)	200	(94.8)

Table8. Compariso between (IgG, IgM) against *Toxoplasma gondii* in patients with cancer in both males and females.

	IgG		IgM	
	Number	**(%)**	**Number**	**(%)**
Positive male	33	(15.6)	1	(0.47)
Negative male	39	(18.5)	71	(33.7)
Positive female	64	(30.3)	10	(4.7)
Negative female	75	(35.6)	129	(61.3)

Chapter 5

- **Discussion**
- **Conclusion**
- **Limitations**
- **Recommendations**

5.1. Discussion

This is probably the first study of prevalence of *T. gondii* infection in cancer patients in Libya. Most of the available studies in Libya had dealt with general epidemiology and other issues including prevalence of *T. gondii* infection among pregnant women.

We found that the seropositivity rate for anti *T. gondii* antibodies in cancer patients was (45.9%) by ELISA. This finding appear to be in accordance with the high prevalence rates of toxoplasmosis reported by most of the previous studies (Kozar, 1953; Roch *et al*., 1953; Li and Jiang *et a l*., 2000; Buratbine *et al.*, 2001 ; Elnahas, 2003; Al-Harthi *et al* ,2006). Our findings are also in accordance with the high prevalence rates of toxoplasmosis in Libya (Legnain *et al*.,1983; ,Khder *et al*., 1987; Gashout *et al.*, 1992; Kassem *et al.,* 1999, Magrhi *et al.*, 2003; Alkateb, 2007; Zaede *et al*., 2007; AL jbale, 2008; Alkhunfas, 2008). This result may indicate that *T. gondii* potentially increases the risk of brain cancers in humans (Thomas *et al.,* 2011).

rates of seroprevalence have also been recorded in many places such as (10.0%) in the United Kingdom by Allain *et al*. in (1998) Esquivel *et al*. in (2006), and in Libya by Abu-Setta and Yamani in (2008). In this study the age group interval 72 > years old showed the highest rate of seropositivity among other age groups (61.5%). This result was statistically significant These results may not be in accordance with results (Legnain, *et al*, 1983; Kassem *et al.,* 1999; Magrhi *et al*., 2003; Alkhunfas, 2008; AL jbale, 2008). However, studies of (Al-Qurashi *et al.,* 2001; Buratbine, 2001) revealed that the risk of acute infection seems to be higher in young ages than in the ages after 30 years, which seem apparently not consistent with the results of this study.

The present study also showed that the seropositive rate of *T. gondii* in female cases was higher than in male patients. The percentage was (30.3%) & (15.6%) in female and male patients respectively. These findings consistent with the results published (Khader, 1987 ; Halder, 1993). they found that the seropositive rate was (41.13%) in females and (17.87%) in males (Halder *et al.,* 1993). It is also not consistent with the results of

(Zbeda, 2009). who found high prevalence rates of toxoplasmosis patients among treated people. Our study showed that the rate of *anti-Toxoplasma* IgM in cancer patients were low (5%), they were in the cases of female (100%).

For this reason, it is important that patients with toxoplasmosis infection are diagnosed and identified in order to refer them for early therapy or other interventions. It is also important to inform and educate these patients on how to avoid possible risk-factors and prevent *Toxoplasma* infection. Hence, it would be desirable that the antibody status of patients be known before, during and after chemotherapy

5.2. Conclusion

In the present study, *T. gondii* prevalence rate in patients with tumours in Tripoli, Libya is (45.9%). Thus, there might be a relationship between tumors and *T. gondii* infection. The results suggest that the infection rate in females is higher than males, The rate of infection in patients Post IgG, treated higher than patients untreated.

5.3. Limitations

It was difficult to collect all required information from such patients as most of them were destressed and depressed and would not have allowed completing the questionnaire. This has prevented obtaining adequate data.

5.4. Recommendations

1- Further studies are necessary to elucidate the relationship between patients infected with *T. gondii* and cancer patients.

2- More focused studies are required to detect the seroprevalence of *T. gondii* infection in the cancer patients who are under treatment.

3- Future investigations should include detection of seroprevalence of *T. gondii* infection and severity of the disease and the type of treatment.

4-According to the prevalence of positive cases in these patients, it is necessary to examine the patients for toxoplasmosis before, during and after chemotherapy

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Chapter 6

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