Ministry of Higher Education and

Scientific Research

University of Babylon

College of Science for Women

Department of Biology

Polymorphism of IL-8 and IL-17A Related with Toxoplasmosis among Pregnant and Aborted Women in Baghdad Province

A Thesis

Submitted to the Council of the College of Science for Women

University of Babylon Partial Fulfillment of the Requirements of

the Degree of Master of Science in Biology

By

Mariam Jbaar Kareem Farhan

B.Sc. Biology, University of Babylon (2017**)**

Supervised by

Prof. Dr.

Ahmed Khudhair Al-Hamairy

2023 A.D 1444 **A.H**

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

«وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ وَسَتُرَدُّونَ إِلَىٰ عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ»

صدق الله العظيم

سورة التوبة (الآية ١٠٥)

Supervisor Certification

I certify that this thesis,entitled (Polymorphism of IL-8 and IL-17A Related with among Pregnant and Aborted Women in Baghdad Province) was prepared by (Mariam Jbaar Kareem) under our supervision at the college of science for women, University of Babylon as a partial requirement for the degree of Master of Science in Biology.

Signature

Name: Dr. Ahmed Khudhair Al-Hamairy

Scientific order: Prof.

Address: College of Science for women\ University of Babylon

Date: \ \2023

In view of the available recommendation, I forward this thesis for debate by the examining committee

Signature

Name: Dr. Ikhlas Mohammed Al-Sharifi

Scientific order : Assist. Prof.

Address : Head of Biology Department College of Science for women\

University of Babylon

Date: \ \ 2023

Dedication

I dedicate this work to:

My dears father and mother.....

My dear husband.....

My beloved children.....

My dear brothers.....

Mariam

Acknowledgments

I start by thanking allah for giving me the strength and patience to complete this study .

I wish to thank the head of the department of Biology and all staff of the Department of Biology/ College of Sciences for Women University of Babylon for helping me .

I would like to foremost express my deepest gratitude to my advisor, Prof. Dr. Ahmed Khudhair Al-Hamairy, for continuous supports and guidance and many thanks to hem for all the great efforts and patience throughout this work .

Special thanks to the nurse Hala Khudhair Khlaf and Najla Zoheer Mahmood at Al- Yarmouk hospital for their help.

Finally, I am very grateful to everyone who supported me to finish this work.

Mariam

Abstract

The current study was aimed to evaluate toxoplasmosis among pregnant and aborted women based on immunological criteria by IgG, IgM, epidemiological criteria and detection polymorphisms of the interleukin-17A and IL-8 genes and discovering its relationship with toxoplasmosis.

Laboratory experiments have been conducted to study 96 samples collected at random from Baghdad Hospital(Al-Yarmok teaching) for pregnant and aborted women in Baghdad province , during the period from October till December 2022 , 96 samples have been examined in VIDAS device for both IgG and IgM, 20 samples were obtained positive for IgG in pregnant and aborted women 14 were for pregnant and 6 for aborted women and no one sample of which is positive for IgM.

According to the division of the study samples into six age groups, that of the total (96)samples pregnant and aborted women, the results show the percentage is(18.5%) in the age groups (18-22) years in pregnant and aborted women patients with toxoplasmosis, the percentage is(18.18%) in age groups(23-27) years in patients with toxoplasmosis, while the percentage is (25%) in the age groups (28-32) years in patients with toxoplasmosis. The highest percentage is (18.75%)in the age groups (33-37) years in patients with toxoplasmosis, while the highest percentage is (26.66%) in the age groups (38-42) years and (0%) in the age groups (43 and more) years in pregnant and aborted women patients had positive IgG antibodies with toxoplasmosis. positive IgM antibodies had(0%) to toxoplasmosis in all age groups. This means the IgG antibodies were

dominant in the current study ,and the absence of the IgM antibody indicated that most of the infection cases were chronic.

According to the division of the study samples according to the residence area in to two residence area (Urban and Rural) the results show the percentage is(31.11%) had positive IgG antibodies in the Urban in pregnant and aborted women patients with toxoplasmosis, the percentage is(11.76%) had positive IgG antibodies in the Rural in pregnant and aborted women in patients with toxoplasmosis. (0%) had positive IgM antibodies to toxoplasmosis in all samples.

According to the division of the study samples of the total (96)samples pregnant and aborted women. According to Cat Presence the division of the study samples according to Cat Presence in to two (Cat Presence and Cat not Presence).The results show the percentage is(21.42%) had positive IgG antibodies to toxoplasmosis in the Cat Presence in pregnant and aborted women patients with toxoplasmosis, the percentage is(20%) had positive IgG antibodies to toxoplasmosis in the Cat not Presence in patients with toxoplasmosis.(0%) had positive IgM antibodies to toxoplasmosis in all samples.

According to abortion No the division of the study samples according to abortion No in to three groups (one time ,two time, three or more time),the results show the percentage is(33.33%) had positive IgG antibodies to toxoplasmosis in one time abortion in aborted women patients with toxoplasmosis, the percentage is(50%) had positive IgG antibodies to toxoplasmosis in the two time abortion in patients with toxoplasmosis and, the percentage is(30%) had positive IgG antibodies to toxoplasmosis in the three time abortion in patients with toxoplasmosis. (0%) had positive IgM antibodies to toxoplasmosis in all samples.

According to duration of pregnancy the division of the study samples according to Duration of pregnancy in to three groups (1-3),(4-6),and(7-9).The results show the percentage is(9.09%) had positive IgG antibodies to toxoplasmosis in(1-3)month in pregnant women patients with toxoplasmosis, the percentage is(20%) had positive IgG antibodies to toxoplasmosis in (4-6)month in pragnany patients with toxoplasmosis and, the percentage is(19.04%) had positive IgG antibodies to toxoplasmosis in(7-9)month in pragnancy in patients with toxoplasmosis.(0%) had positive IgM antibodies to toxoplasmosis in all samples.

This means the IgG antibodies were dominant in the current study ,and the absence of the IgM antibody indicated that most of the infection cases were chronic.

The results of the genotyping of the wild and mutated alleles of the single nucleotide polymorphism of the interleukin-17A and IL-8 gene and evaluation of their relationship to toxoplasmosis in pregnant and aborted women. It was found that the presence of two SNPs in the promoter region of the genes of interest are rs2275913 SNP to IL-17A and rs4073SNP to IL-8. The homozygous AA mutant genotypes show greater distribution in toxoplasmosis and pregnant and aborted women patients compared to the control group, odds ratio (OR) values are supporting that the A allele in AA is associated with the disease of interest and is considered a risk allele. The odds ratio is higher in genotype AA=1.7(0.40-7.3)with a highly significant p-value of 0.4, and the frequency of the allele A is higher =12(30%)in the group of patients with a high OR=1.71 (0.61-4.8) and P-value=0.3 while the allele frequency is 8(20%) low in the control group. Multiple chromatogram alignment results of toxoplasmosis and pregnant and aborted women

patients show three genotypes, GG, AG and AA. The A allele is considered a mutated allele based on the correct SNP rs2275913. The AG and AA genotypes show a greater distribution in toxoplasmosis and pregnant and aborted women patients are compared to the control group. Odds ratio (OR) values support that the A allele in both AG and AA is associated with the disease of interest. The OR is higher in the genotypes AG=1(0.019-52.03), P-value=1 and AA=1.7(0.40-7.3)with a highly significant P-value of (0.4).The similar results to IL-8gene.

The final result shows that the analysis of interleukin-17A andIL-8 polymorphism has a clear effect on any human diseases. In this study, the interleukin-17A and IL-8 polymorphism shows high efficacy in the occurrence of toxoplasmosis and pregnant and aborted women, and shows the role, polymorphism and distribution of interleukin-17A for genotypes rs2275913 G>A and interleukin-8 rs4073 A>T alleles are not similar between patients and control.

List of Contents

Contents		Page No.
Abstract		I-IV
List of Contents		V-IX
List of Tables		IX-XI
List of Figures		XI-XII
List of Pictures		XIII
List of Abbreviation		XIII-XIV
List of Appendixes		XVI
NO.	Chapter One: Introduction	
1-1	General Introduction	1-3
1-2	The Aim of the Present Study	4
Chapter Two: Literatures Review		
2-1	Historical View of Toxoplasmosis	5
2-2	Classification of *Toxoplasma gondii*	6
2-3	Morphology of *T. gondii*	6
2-3-1	Tachyzoites	6

2-3-2	**Bradyzoite or Tissue Cysts**	**7**
2-3-3	**Oocyst**	**8**
2-4	**Life Cycle of *Toxoplasma gondii***	**10**
2-5	**Modes of transmission of toxoplasmosis**	**12**
2-6	**Epidemiology of Toxoplasmosis**	**13**
2-6-1	**Toxoplasmosis in the Worldwide**	**13**
2-6-2	**Toxoplasmosis in Arab World**	**14**
2-6-3	**Toxoplasmosis in Iraq**	**15**
2-7	**Pathogenicity**	**17**
2-8	**Immunology of *Toxoplasma gondii***	**19**
2-9	**Immune response in toxoplasmosis**	**20**
2-10	**Diagnosis of Toxoplasmosis**	**21**
2-10-1	**Direct methods**	**21**
2-10-2	**Immunological diagnosis**	**21**
2-10-3	**Molecular diagnosis**	**22**
2-11	**Interleukin-8**	**23**
2-12	**Interleukin-17A**	**24**

Chapter Three: Materials and Methods		
3-1	The Equipment's and Tools	27
3-2	Biological and Chemical Materials	28
3-3	Experimental Design of the Study	30
3-4	Methods	30
3-4-1	Samples Collection	30
3-4-2	Blood Samples Collection	31
3-5	Immunological assay	32
3-6	Molecular assay	38
3-6-1	The extraction of DNA from blood	38
3-6-2	Estimation the DNA concentration and purity	39
3-6-3	Primers	39
3-6-4	Dissolving of Primers	40
3-6-5	Prepared the Reaction Mixture	40
3-6-6	Thermal Cycling Conditions	41
3-7	Validity of the IL-17a Genotype	41
3-8	Detection of Amplified Products by Agarose Gel Electrophoresis	42

3-9	**Interpretation of Sequencing Data**	**42**
3-10	**Statistical Analysis**	**43**
Chapter four: Results and Discussion		
4-1	**Epidemiological Criteria**	**44**
4-2	**Polymorphism of genes**	**56**
4-2-1	**locations of IL-8 and flanking region of primers**	**56**
4-2-2	**locations of IL-17A and flanking region of primers**	**57**
4-3	**DNA extraction results for IL-8**	**57**
4-4	**DNA extraction results for IL-17A**	**59**
4-4-1	**Amplification of partial region of IL17A gene**	**60**
4-5	**Sequence results forIL-17A**	**61**
4-6	**The multiple alignments of chromatograms T. gondii with aborted and pregnant women.**	**62**
4-6-1	**The multiple alignments of chromatograms of patient cases**	**62**
4-6-2	**The multiple alignments of chromatograms of control group**	**63**
Conclusions and Recommendations		
Conclusions		**67**

Recommendations	**68**
References	**69-87**
Appendices	**88-90**
الخلاصة بالعربي	**أ- ج**
العنوان بالعربي	

List of Tables

Table No.	Title	Page No.
3-1	The Equipment's and Tools used in the Current Study	27
3-2	Biological Materials and Chemical Material used in the Current Study	28
3-3	Content of The Kit	33
3-4	Description of the TXM strip	35
3-5	The primer IL-8 pair was used in the current study	39
3-6	The primer IL-17A pair was used in the current study	40

3-7	The final volume of 25µl PCR reaction compone	41
3-8	Thermal cycling conditions for specific primers for IL-17A and IL-8 gene under interest.	41
4-1	Percentage of infection of toxoplasmosis in the pregnant and aborted women by using the vidas technique	44
4-2	Percentage of infection toxoplasmosis in the pregnant and aborted women by using the vidas technique according to the Age(year).	46
4-3	Percentage of infection toxoplasmosis in the pregnant and aborted women by using the vidas technique according to the residence area	48
4-4	Percentage of infection toxoplasmosis in the pregnant and aborted women by using the vidas technique according to Cat Presence	50
4-5	Percentage of infection toxoplasmosis in the aborted women by using the vidas technique according to Abortion No.	52
4-6	Percentage of infection toxoplasmosis in the pregnancy women by using the vidas technique according to Duration of pregnancy	54
4-7	Genotypes number and allele frequency of SNP rs4073	59

	of IL-8 between patients and control groups ,shown P-value (0.05) and the Odd Ratio values	
4-8	Genotypes number and allele frequency of SNP rs2275913 of IL17A between patients and control groups ,shown P-value (0.05) and the Odd Ratio values.	64

List of Figures

Fig. No.	Figure	Page No.
2-1	*Toxoplasma gondii* (A) Tachyzoites (B) Bradyzoites (C) Oocyst	9
2-2	*Toxoplasma gondii. Trophozoite (tachyzoite)*	9
2-3	*Toxoplasma gondii* Life cycle	11
3-1	Experiment design of the study	30
4-1	The rs4073 located on Chromosome 4	56
4-2	The SNP rs2275913 located on chromosome 6 Figure	57
4-3	IL-8 gene polymorphism of patients group	57
4-4	IL-8 gene polymorphism of control group	58
4-5	Gel-electrophoresis illustration quality of DNA extracted from patients group	59

4-6	Gel-electrophoresis illustration quality of DNA extracted from control group	60
4-7	Gel electrophoresis 52185415-52186396 as target DNA region of IL17A amplification region with flanking primers from patient group	60
4-8	Gel electrophoresis 52185415-52186396 as target DNA region of IL17A amplification region with flanking primers from control group	61
4-9	The results of targeted region shown one SNP: rs2275913 G>A from patient group	62
4-10	The results of targeted region shown one SNP: rs2275913 G>A from control group	63

List of Pictures

Picture No.	Pictures	Page No.
3-1	The vidas technique	32

List of Abbreviations

Term	Meaning
Ab	Anti body
Bp	Base pair
°C	Centigrade
CSIF	Cytokine Synthesis Inhibitory Factor
CKS	Chemokines
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay

Fl	Femtoliter
g/dl	gram/deciliter
HIV	Human immune deficiency virus
IFA	Immunofluorescence Assay
IFI	Indirect immunofluorescence
IgG	Immunoglobin G
IgM	Immunoglobin M
IL-17A	Interleukin-17A
IL-8	Interleukin-8
INF	Interferon
IU	International Unite
kDa	Kilodalton
LAT	Latex Agglutination Test
LAMP	Loop Mediated isothermal amplification
µl	Microliter
Mm	micrometers
MAT	Modified Agglutination Test
mg/dl	milligram/ deciliter
Ml	Milliter

NCBI	National Center for Biotechnology Information
ng/µl	Ninogram/microliter
OD	Optical Density
OR	Odd Ratio
OS	Oxidative Stress
PBMC	Peripheral Blood Mono-nuclear Cells
PCR	polymerase Chain Reaction
PV	Parasitophorus Vacuoles
RFLPs	Restriction Fragment Length Polymorphisms
Rs	Reference strain
RT	Retention Time
Se	Standard Error
SPR	Solid phase receptacle
SNPs	Single Nucleotide Polymorphisms
SPSS	Statistical Package for the Social Sciences
SPR	Solid Phase Receptacle
T. gondii	*Toxoplasma gondii*
TGF	Transforming Growth Factor
USA	United States of America

List of Appendices

Number	Subject	Page
(1)	Questionnaire sheet that provided for all patients in the present study.	87
(2)	The ethical paper given to pregnant and aborted women patients to take agree or not to take blood samples.	88
(3)	A letter facilitating a task addressed to Baghdad Hospital(AL- Yarmouk).	89

Chapter One
Introduction

Chapter One

1- Introduction

1-1: General Introduction

Toxoplasma gondii, the parasite protozoan that causes toxoplasmosis, is an obligate intracellular parasitic with noteworthy zoonotic, causes toxoplasmosis that infects in humans and warm-blooded animals(Ybanez *et al*.2020) . *T. gondii* is transmitted mostly through the eating of undercooked meat and fish containing bradyzoites are rotes for toxoplasmosis. Also vegetables, water and milk contaminated with oocysts and transfusion and transplantation of blood and organ, harboring tachyzoites from patients infection are major sources of *T.gondii* infection in humans(Koloren and Dubey , 2020).

Toxoplasmosis, by instance, affects approximately a third of the world's population (Flegr *et al*., 2014). More worrisome, Montazeri *et al*. (2020) indicated that 35% to 76% of wild and domestic felids are infected with *T. gondii*, implying that *T. gondii* infection in humans and animals is on the rise globally, and that more research is needed to combat these parasite infections. Management, prevention, and therapy with safe and efficient pharmaceutical inhibitors are all necessary measures, even though there are some medications capable of treating *T.gondii* infections in humans and other animals, they have drawbacks such as toxicity, high cost, and more importantly, most drugs are unsuccessful in treating the latent stage (bradyzoite) that persists in the brain (Shiojiri *et al*., 2019; Angel *et al*., 2020; Secrieru *et al*., 2020).

Pregnancy is one of the most critical steps in women's lives, particularly those who want to become a mother for the first time.

Abortion is a problem that any women might experience during pregnancy, and therefore suffer from psychological issues and medical expenses, which make it particularly important. One of the reasons for abortion is toxoplasmosis, which is due to an infection caused by *Toxoplasma gondii*, an obligate intracellular parasite, belonging to the phylum of Sporozoa, causing toxoplasmosis disease in humans and most of the warm-blooded animals around the world(Duby.,2004).

Cytokines are important mediators in the bi-directional interaction between the maternal immune system and the reproductive system during pregnancy(Mohammed *et al.*, 2012) . Interleukin-8 (IL-8) and interleukin-17 (IL-17) are pro-inflammatory cytokines produced by several tissues upon the inducement of a number of factors, among which are membrane LPS from gram negative bacteria, Viruses and several cytokines, their action is directed towards either myeloid or non-myeloid cellular targets (Mekori and Metcalfe.,2000). Interleukin 8 (IL-8) is a chemokine produced by macrophages and other cell types such as epithelial cells. It is also synthesized by endothelial cells, which store IL-8 in their storage vesicles. IL-8, also known as neutrophil chemotactic factor, has two primary functions. It induces chemotaxis in target cells, primarily neutrophil but also other granulocytes, causing them to migrate toward the site of infection. Endometrium also produces IL-8 that is abortogenic. Mast cells are essential for inflammation by liberating several multifunctional cytokines including IL-8.If a pregnant mother has high levels of interleukin-8, there is a

Genetic polymorphism, the definition by Cavalli-Sforza and Bodmer, is the occurrence in the same population of two or more alleles at one locus, each with appreciable frequency, where the minimum frequency is typically taken as 1% (Philip *et al.*,2013). An allele is one of the variant

forms of agene at specific locus on a homologous chromosome. The different forms of the polymorphism (alleles) are observed more often in the general population than mutations. The most common polymorphism in the human genome is the Single-Nucleotide Polymorphism (SNP) (Crawfordand Nickerson.,2005).

1-2:The Aim of the Present Study

The present study aims to evaluate the relationship of IgG and IgM in toxoplasmosis among pregnant and aborted women patients, based on age and cat presence and detecting the role of polymorphism of promoter region of gene IL-8 and IL-17A based of genotyping mutant alleles in one SNP in pregnant and aborted women with *T.gondii* and *T.gondii* alone, compared with control group based on the following objectives:

1- Detecting the positive toxoplasmosis in pregnant and aborted women patients by using immunological vidas device for IgG and IgM.

2- Determining the relationship between positive toxoplasmosis and some epidemiological criteria (age groups, residence area, presence or absence of cats,duration of pregnancy,and abortion No.).

3- Estimation the genotyping, allele frequency based evaluated the Odd Ratio (OR) on polymorphism of IL-8 andIL-17A in patients pregnant and aborted women that infected with toxoplasmosis and comparing them with control group.

Chapter Two
Literatures Review

CHAPTER TWO

2- Literatures Review

2-1: Historical View of Toxoplasmosis

Toxoplasma gondii was first described in 1908 by Nicolle and Manceaux in Tunisia, and independently by Splendore in Brazil **(Ferguson, 2009)**. Splendore reported the protozoan in a rabbit, while Nicolle and Manceaux identified it in a North African rodent, the gundi (*Ctenodactylus gundi*) **(Weiss and Dubey, 2009)**.The first recorded case of congenital toxoplasmosis was in 1923, but it was not identified as caused by *T. gondii*. Janků (1923) described in detail the autopsy results of an 11-month-old boy who had presented to hospital with hydrocephalus **(Weiss and Dubey, 2009)**.In 1948, a serological dye test was created by Sabin and Feldman based on the ability of the patient's antibodies to alter staining of Toxoplasma. Transmission of Toxoplasma by eating raw or undercooked meat was demonstrated by Desmonts *et al*. in 1965 Paris **(Ferguson, 2009).** In 1974, Desmonts and Couvreur showed that infection during the first two trimesters produces most harm to the fetus**(Weiss and Dubey, 2009).** Toxoplasmosis is known to be one of the most common infections worldwide in humans and warm-blooded animals. One-third of the world's population is infected with toxoplasmosis **(Halonen and Weiss, 2013)**.

2-2: Classification of *Toxoplasma gondii*

According to Hill and Dubey (2014), *T. gondii* is classified as follows:

Kingdom: - Protista

Class: - Sporozoasida

Sub class: - Coccidiasina

Order: - Eucoccidiida

Sub order: - Eimeriidae

Family: - Toxoplasmatidae

Genus: - *Toxoplasma*

Species: - *gondii*

2-3: Morphology of *T. gondii*

2-3-1: Tachyzoites

The different phases of development of *Toxoplasma gondii* show important changes in its morphology, these are called: Tachyzoites, Bradyzoites and Oocysts.The tachyzoite is the form of acute infestation, it is (4-8) micrometers (μm) in length and (2-4) micrometers (μm) in width, with the appearance of a crescent as shown in figure [2.1 (a) and (b)]. A group of molecular structures, at one end of the Tachyzoite of *T. gondii*, with the denomination of apical complex, is fundamental for both the invasion and the proliferation of this parasite **(Gómez-De León and Mondragón-Flores, 2017).**

Tachyzoite cell division is rapid, generating two new cells per mother cell every 6–8 h. **(Knoll *et al.*, 2013).**The tachyzoite is a highly proliferative

form commonly associated with acute infection, reactivation and vertical transmission(**Sinai *et al*., 2017; Cerutti *et al*., 2020**).

Tachyzoite, although not a resistant stage, plays an important role in *Toxoplasma* epidemiology if primary infection occurs during gestation, in which case there is a high probability of transmission to the fetus **(Robert-Gangneux and Dardé, 2012 ; Dubey *et al*., 2020).**

2-3-2: Bradyzoite or Tissue Cysts

The bradyzoite, on the other hand, is a latent, albeit metabolically active, slow growing encysted form **(Sinai *et al*., 2017; Cerutti *et al*., 2020).**Bradyzoites are slimmer than tachyzoites and present solid rhoptries,which are labyrinthine in tachyzoites, along with many amylopectin granules that are fewer absent in tachyzoites as illustrated in [figure 2.1 (b)] (**(Ferguson and Dubremetz, 2014).**

Bradyzoites divide slower than tachyzoites but can assemble and sustain between 1000–2000 bradyzoites per cyst **(Knoll *et al*., 2013).**Bradyzoites are commonly associated with the chronic stages of toxoplasmosis, persistence, immune evasion, and are refractory to currently available anti-toxoplasmosis pharmacotherapies (**Sinai *et al*., 2017; Cerutti *et al*., 2020**).Because of their ability to persist for a long time in the intermediate hosts, bradyzoites play a central role in the life cycle of the parasite. The classical completion of the cycle includes predation of an intermediate host by felids, and thus ingestion of parasite-containing tissue cysts. They then differentiate into sexually-competent stages that lead to the formation of the oocysts that are shed by the felid in the environment to start another cycle **(Dubey, 2014).**Bradyzoites are also located inside a PV which, however, over time matures to a so-called intracellular parasitophorus vacuole. The most prominent feature of tissue cysts is the development of a thick cyst wall that is thought to confer

structural rigidity **(Tomita *et al*., 2013).**Both tachyzoites and bradyzoites follow a cell division scheme known as endodyogeny consisting of a single round of DNA replication by semi-closed nuclear mitosis. The internal assembly of two daughtecells occurs concomitantly with nuclear mitosis, inside the mother cell **(White and Suvorova, 2018; Gubbels *et al*., 2020)**.

2-3-3: Oocyst

Synthesis of the oocyst wall begins in the macrogamont, which possesses wall-forming bodies , Sporulated oocysts [figure 2.1 (c)], are highly resistant in the environment and to chemical inactivation agents; therefore, prevention of oocyst shedding is a major factor in lowering possible infection sources **(Shapiro *et al*., 2019).** After fertilization, the unsporulated oocyst is formed with a single sporont, During sporulation, the sporont forms two sporocysts, each containing four sporozoites, originating the sporulated oocyst **(Ferguson and Dubremetz, 2014 ; Attias *et al*., 2020).**Oocyst ingestion explains infections in vegetarians and herbivore species, however, this transmission routes not limited to these groups. A recent meta-analysis study reported a mean pooled prevalence of *T. gondii* oocysts in public environments of 16%, ranging from 8% to 23% in the sampled continents, North America, Asia, South America and Europe. Felids are able to shed millions of oocysts upon infection, acting as disseminators of the sporozoite infective stage **(Maleki *et al*., 2021).**

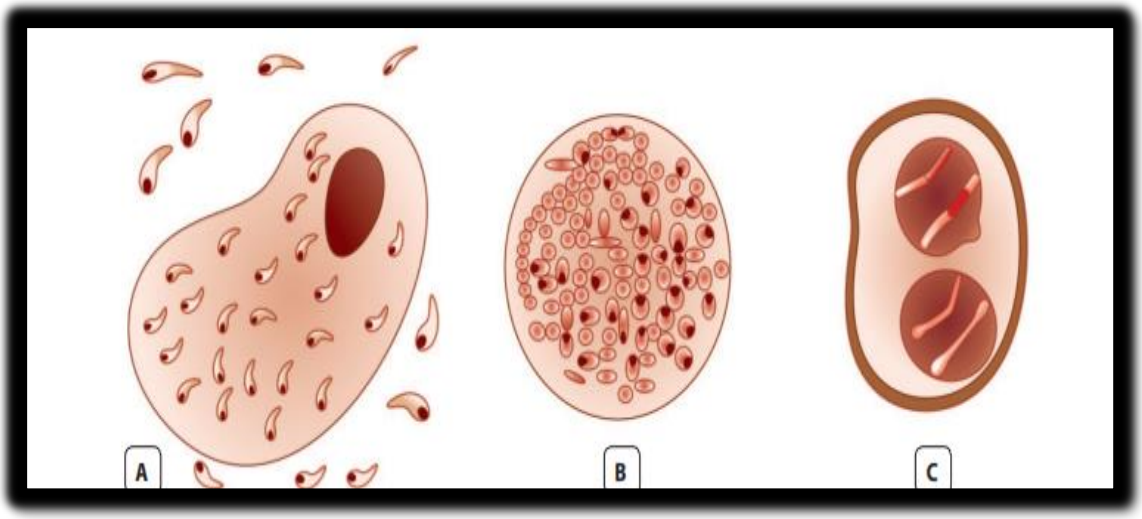


Figure (2-1): *Toxoplasma gondii,* A. crescent tachyzoite –extracellular trophozoites and intra cellular from within magrophage: B. Thick walled tissue cyst containing round forms – bradyzoites: C. Oocyst containing 2 sporocysts within sporozoites inside (Ghosh, 2013).

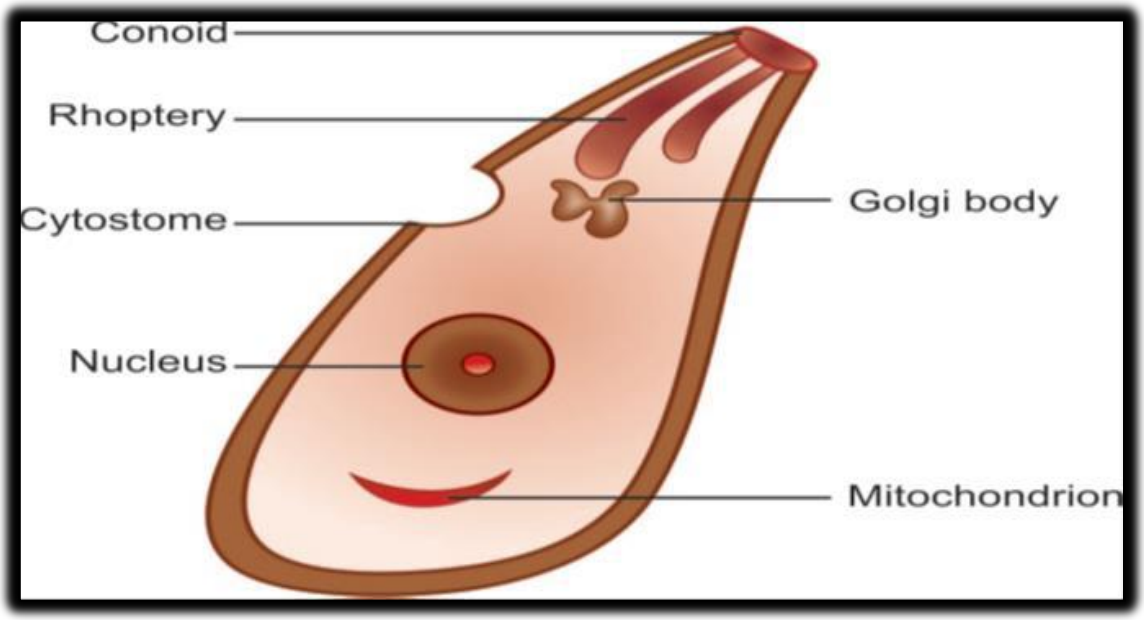


Figure (2.2): *Toxoplasma gondii. Trophozoite (tachyzoite)* (Ghosh, 2013).

2-4: Life Cycle of *Toxoplasma gondii*

T. gondii has a complex life cycle that includes an asexual and sexual cycle; the asexual cycle takes place in a wide spectrum of intermediate hosts, but the sexual cycle occurs exclusively in feline hosts (cats), who shed infectious oocysts in their feces (Gissot, 2022).

The tachyzoite, a highly versatile, asexual stage of *T. gondii*, is capable of adhering, invading, altering, and multiplying rapidly within a variety of nucleated cells, allowing it to infect a wide range of warm-blooded intermediate hosts (Dubey, 2014). Intermediate hosts produce strong immune responses, causing tachyzoites to transform into bradyzoites, which are long-lived, asexual, and contagious parasites that live in cysts within cerebral and muscle cells (Miller *et al*., 2009).

T. gondii is only found in felids (cats) that serve as final host and develops through a conventional coccidian life cycle (Ferguson and Dubremetz, 2014). Predation of an infected intermediate host by a definitive host results in bradyzoite attack of enterocytes, going to initiate several rounds of quick asexual reproduction, generating merozoites, accompanied by transformation into male (micro-) and female (macro-) gametes in the epithelia of the small intestine, in addition to spreading systemically as in intermediate hosts (Yongzhen *et al*., 2018).

Fertilization of macrogametes by microgametes produces zygotes, the only one diploid stage of the life cycle. Resistant bi-layered walls grow around the zygotes, resulting in oocysts. The latter is initially unsporulated and non-infectious, but with enough temperature, moisture, and oxygen. It will perform meiosis for sporulate, generating two sets of four haploid sporozoites, which are housed inside a second set of walled structures known as sporocysts (Gazzinelli *et al*., 2014).

Although these sporulated oocysts are infectious, it should be emphasized that there is some debate over whether fertilization is required for the production of sporulated oocysts (Walker *et al*., 2015).

Furthermore, cats producing oocysts in farming communities infect cattle and cause microscopic, infectious cysts in tissues. When all of these data are considered, it is clear that human exposure to *T. gondii* infectious stages is common whether via oocysts or tissue cysts. It is still debatable whether route of infction is more essential, but it is known that every human infection is caused by cats releasing oocysts into the surroundings, either directly or indirectly see Figure (2-2) (Hussein *et al*., 2017).

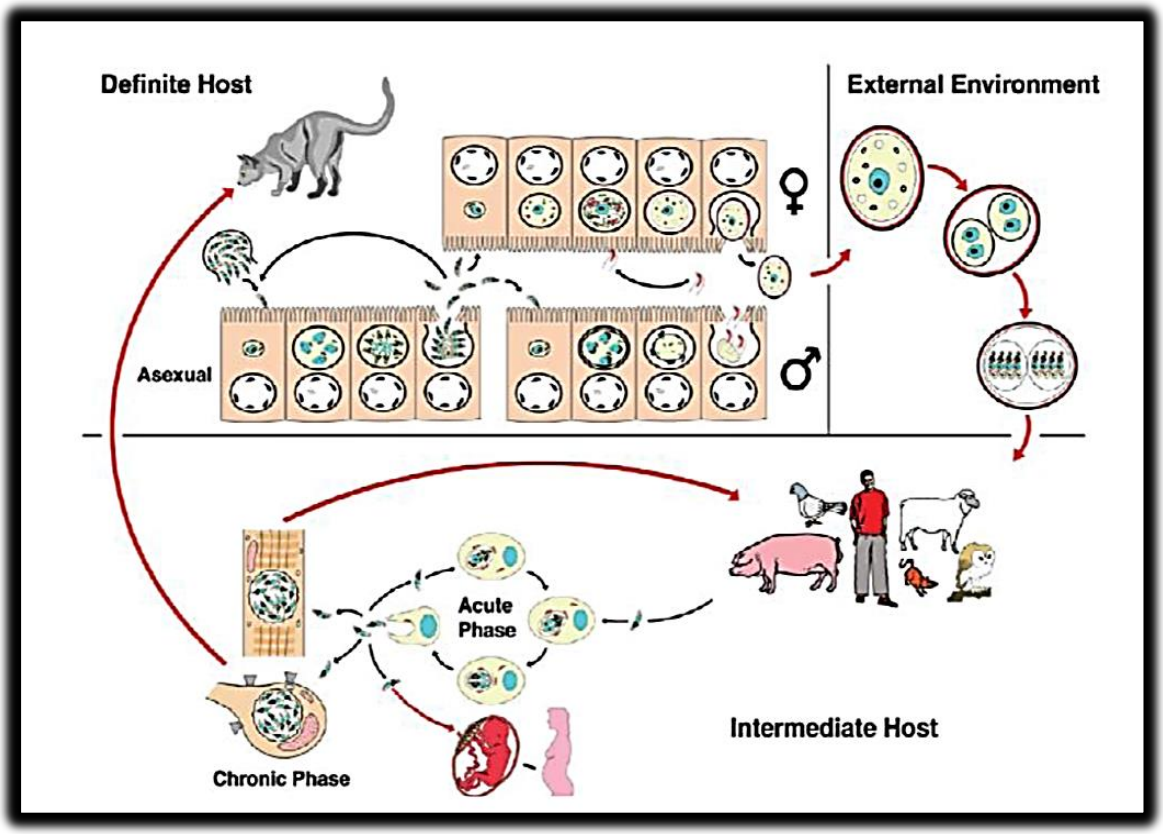


Figure (2-3) Summary of the life cycle of *T. gondii* (Ferguson, 2009).

2-5: Modes of transmission of toxoplasmosis

Toxoplasmosis is spread in a variety of ways, as shown below:

1. During consumption of raw or undercooked meat containing bradyzoites or formed tissue cysts, including such sheep, cows, and chickens (Mahami *et al*., 2017). Working in the dairy business, as well as meat packing employees and those who sell meat, creates a risk for contracting this parasite (Amissah, 2020).
2. Fresh fruits and vegetables that have not been properly cleaned and are infected with infective phase oocysts are consumed (Shapiro *et al*., 2019).
3. Transmission from an infected woman to her fetus via the placenta (Omidian *et al*., 2021).
4. Through oocysts in polluted water and soil (Pinto-Ferreira *et al*., 2019). When someone eats an oocyst, he will get infected. This oocyst is resistant to difficult environmental living conditions and can survive in contaminated soils for further than one year (Gotteland *et al*., 2014).
5. Animal milk and bird eggs could be a cause of toxoplasmosis infection to humans. Many animals, especially sheep, goats, cows, rats, and cats, have acquired tachyzoites phase (Stelzer *et al*., 2019).
6. During transfusions of blood and organ transplants (Manouchehri *et al*., 2019).

2-6: Epidemiology of Toxoplasmosis

Toxoplasmosis is a worldwide infection that affects 500 million people, with an estimated affected (12-90 %), and increase with age, low education, overcrowding, health, social, economic, traditions and ethnic issues, intake of undercooked meat, exposure to cat excrement, and immune impaired individuals more susceptible to infection were all factors (Gaetano *et al*., 2010). It can boost all of these elements, as well as climate circumstances, to create antibodies against parasites. As a result, oocysts can thrive in warm, wet soil from heated, dry places (Jones *et al*., 2006; Petersen, 2007).

2-6-1: Toxoplasmosis in the Worldwide

According to recent seroprovenance data, *T. gondii* prevalence in the Korean population ranges from 0.97% to 12.9%, with latitudes fluctuating in the south of Korea from 1960 to 1999, in Korea, it affects residents, children, and patients in hospitals at a lower rate, spanning from 1.1% to 7.7%, as compared to American and European locations (Tenter *et al*., 2000).

In the United States Jones *et al*. (2001) reveal that majority of the published statistics on seroprevalence are among women of reproductive age and/or those who are pregnant. According to information from 88 countries, the parasite seroprevalence rises with age in the majority of human populations, and it varies by sexual identity.

Toxoplasmosis is a major public health issue in Brazil, with a serological frequency of 50-54% in adulthood (Bahia-Oliveira *et al*., 2003). Toxoplasmosis prevalence was researched in several countries of the world, with the highest rate found among pregnant women in France, spanning from 87% in 1984 (Hegab and Al-Mutawa, 2003).

On the other hand, the high prevalence rate of the indian population, Malaysia, and Nepal has been recorded. Toxoplasmosis IgG seroprevalence was 45 % overall. Just seven women (3.3%) had IgM antibodies, but only two of these had low IgG avidity, indicating a recent infection of less than four months (Singh and Pandit, 2004). The infection had indicated *T. gondii* consider the expansion in regions competent Iran and seropositivity 5.7 through 78%, according to reports (Ali *et al*., 2007).

Around 88% of the population in France is infected, owing to widespread ingestion of enormous amounts of raw and minimally cooked meat (Larosa *et al*., 2008), however, from 2000 to 2009, it exhibited a modest increase in toxoplasmosis seroprevalence (Shin *et al*., 2009).

In 2008, the Iranian Ministry of Health reported that the rates of toxoplasmosis were 40.7% in Isfahan, 44.2% in Lorestan, and 34.2% in Bandar-e-Abbas (Shin *et al*., 2009). In another study, pregnant women utilizing IFA in Chaharmahal and Bakhtyari, Iran, had a seroprevalence of *T. gondii* antibodies of 27.6% (Ebrahimzadeh *et al*., 2013). In Pakistan, Sadiqui *et al.* (2018) state that among 500 pregnant women, the total seroprevalence of toxoplasmosis was 24.8% and the percentage infection for acute infection (IgG) was 8%.

2-6-2: Toxoplasmosis in Arab World

Many studies have been conducted at the national level of Arab nations to assess the seroprevalence rates of parasite infection, Elnahas *et al.* (2003) declare that anti-*Toxoplasma* antibodies were detected in 34.1% of Sudanese people in a research. Another study is carried out by Al – Qurashi (2004) which states that a high *T. gondii* rate of 52.1% was recorded among donors in the Asir region of Saudi Arabia, and Nimri *et*

al. (2004) shows in a study conducted on 148 women in Jordan who have had a previous pregnancy is normal and ranged percentages recorded for my body antibody IgG and IgM between 45% and 27% for chronic and acute infection, respectively.

Also Jumaian (2005) shows that toxoplasmosis is shown to be prevalent in 31.6% of pregnant women in one study, and 53.1% in another one in Kuwait by (Iqbal and Khalid, 2007). Just a few researches have been carried out in Saudi Arabia to determine the prevalence of *T. gondii*, including investigations in Riyadh, the eastern area, and Jeddah (Tonkal, 2008). In Qatar, Abu-Madi and Haydee (2010) explain that the rate of toxoplasmosis infection amongst 823 women is 35.1% for IgG and 5.2% for IgM, respectively.

2-6-3: Toxoplasmosis in Iraq

In Mosul, Al-Obeidi (2004) makes a study on a total of 406 patients with serum; three distinct laboratory procedures have been used to diagnose a positive serum. A latex test revealed active infection in 79% of cases, followed by a dye test in 46 % of cases, and an ELISA test in 37% of cases.

Razzak *et al*. (2005) in the Kirkuk province, states that the infection rates are found to be 61.2% among pregnant women and 33.3% among non-married women. Also a study in the province of Dohuk by Razzak *et al*. (2005) using the ELISA technique, it is discovered that the infection rate among women with a single fetus or many losses is 0.97%. Yacoub *et al.* (2006) in Basrah, the infection rate amongst aborted women is reported to be 59.1%.

Al Dalawi (2007) explains that by utilizing mini VIDAS technique, researcher discovers that the proportion of illness

toxoplasmosis of IgG is 29.2% in Baghdad. Kareem (2008) concentrates on using the ELISA technology, it is discovered that the rate of infection in Baghdad is 31.04 % amongst previously and suddenly aborted women, apparently healthy pregnant women, and apparently healthy non married women. Khalil (2008) in Baghdad, on the other hand, explains that by using ELISA method, it is discovered that the prevalence of acute toxoplasmosis disease among women had been reduced by antibody immunoglobulin (21.5%).

Al-Rawi (2009) indicates that 4.16% of aborted women with IgM antibodies have toxoplasmosis, while 25.83% has IgG antibodies. In Baghdad, by using the ELISA technology, it is 13.33% for both (IgM and IgG). Al-Shikhly (2010) explains that by using the latex agglutination test (LAT), ELISA IgM and IgG procedures, the rate of toxoplasmosis among healthy females in Baghdad is 51.52%, 41%, and 16.2%, respectively. In Iraq, the parasite is expanded to 36.6% in Basrah, 58% in Kirkuk, and 29.2% in Tikrit in 2011 (Al-Dory, 2011; Al-Mousawi and Shani, 2011; Kadir *et al*., 2011).

In Kut Al-Mayahi (2011) states that by using the ELISA IgM and IgG techniques, researchers discover that the incidence of toxoplasmosis in women is 66.35% and 44.71%, respectively. In Babylon province, Al-Mosawi (2012) declares that by using the ELISA technology, it is discovered that the rate of toxoplasmosis amongst pregnant women infected with diabetes for IgG, IgM, and both (IgG, IgM) is 69.1%, 17.4%, and 48.8%, respectively. In Babylon province, Al-Ghargholi (2014) says that by using the ELISA IgM and IgG techniques, researchers discover that the rate of *T. gondii* infection in diabetes mellitus patients is 51.4% and 11.4%, respectively.

In Baghdad province, Al-Rawazq (2017) explains that the seroprevalence of *T. gondii* IgG antibodies resulted 63.4% of 110 pregnant women being examined. In Tikrit province, Tawfiq *et al*. (2019) state that IgG-*Toxoplasma* is found to be positive in 35.5% of pregnant women in the context of congenital or acquired toxoplasmosis. As with the brain and muscles, the eyes are one of the organs the tachyzoites can disseminate to upon initial infection. There, they can cause self-limiting lesions, but might encyst and be able to subsequently reactivate if host immunity becomes impaired.

In addition to the obvious deleterious effects of acute toxoplasmosis, chronic toxoplasmosis (i.e. the long-term persistence of the parasite in the form of tissue cysts), especially as it targets the central nervous system, may also have an important impact on behavioral changes and psychiatric disorders **(Tyebji *et al*., 2019).**

Epidemiological studies and meta-analyses have shown that *T. gondii* seropositivity can be associated with a number of mental health disorders, including schizophrenia, but also epilepsy and neurodegenerative diseases **(Ngô *et al*., 2017),** although causality has not been firmly established

2.7: Pathogenicity

The type of *T. gondii* strain also has a considerable impact on the pathogenicity, with, for example, severe cases of acquired toxoplasmosis in immunocompromised patients caused by highly pathogenic South American strains from the wild and pation who take immune-suppressive drug(Galal *et al*.,2019).

One of the most critical factors influencing susceptibility to *T. gondii* remains the host immune system and the way it is modulated by parasite factors **(Tomita *et al*., 2021).**

In adults, immunodeficiency can also lead to severe toxoplasmosis, which is most often the result of reactivation of latent infection, even if acute acquired infection may also occur. Individuals who are immune-compromised or immunosuppressed (in the context of HIV infection **(Wang *et al*., 2017),** or for cancer patients and transplant recipients) are particularly at risk **(Ali *et al*., 2019)**.

The most serious outcome in this context is arguably toxoplasmic encephalitis, in which recurrence of toxoplasmosis from parasites encysted in the central nervous system can lead to substantial tissue damage and inflammation **(Blanchard *et al*., 2015).**

Congenital toxoplasmosis can occur when there is primary maternal infection during pregnancy, as during the parasite dissemination phase it may cross the placental barrier to contaminate the developing fetus. It can cause neurological, ocular, or systemic damage with variable severity, which depends on the gestational age at the time of primary maternal infection. For instance, first-trimester maternal infection can lead to more severe manifestations **(Syrian and Sébastien, 2021).**The most important sequelae for the newborn include hydrocephalus, mental retardation, epilepsy, and blindness, although some of these can also occur later in life **(McAuley, 2014)**.

There is also an ocular presentation of the disease called ocular toxoplasmosis, a progressive necrotizing retinitis, that may lead to vision-threatening complications **(Park and Nam, 2013).** This can happen both

in the context of congenital or acquired toxoplasmosis. As with the brain and muscles, the eyes are one of the organs the tachyzoites can disseminate to upon initial infection. There, they can cause self-limiting lesions, but might encyst and be able to subsequently reactivate if host immunity becomes impaired.

In addition to the obvious deleterious effects of acute toxoplasmosis, chronic toxoplasmosis (i.e. the long-term persistence of the parasite in the form of tissue cysts), especially as it targets the central nervous system, may also have an important impact on behavioral changes and psychiatric disorders **(Tyebji *et al*., 2019).**

Epidemiological studies and meta-analyses have shown that *T. gondii* seropositivity can be associated with a number of mental health disorders, including schizophrenia, but also epilepsy and neurodegenerative diseases **(Ngô *et al*., 2017),** although causality has not been firmly established.

2-8: Immunology of *Toxoplasma gondii*

Protective immunity to *T. gondii* involves both the innate and the adaptive immune responses **(Sasai and Yamamoto, 2019).** It is dominated by antibody production against parasitic antigens, and is primarily dependent on T helper 1 cell-mediated immunity, which is characterized by high levels of interleukin-12and Cytokines are important mediators in the bi-directional interaction between the maternal immune system and the reproductive system during pregnancy**(Mohammed *et al*., 2012)** . Interleukin-8 (IL-8) and interleukin-17 (IL-17) are pro-inflammatory cytokines produced by several tissues upon the inducement of a number of factors, among which are membrane LPS from gram negative bacteria, viruses and several cytokines, their action is directed towards either myeloid or non-myeloid cellular targets **(Mekori and Metcalfe.,2000)** . Besides controlling the

acute infection, these cytokines also induce tissue cyst formation of the slowly replicating bradyzoites and thus enable sustained latent infection **(Hunter and Sibley, 2012)**. Recrudescent infection can occur if the immune status of the host is compromised, resulting in conversion of bradyzoites back to the tachyzoite stage. *T. gondii* actively infects host cells and resides in special membranous structures called parasitophorous vacuoles (PVs), where *T. gondii* is able to proliferate. If the PV is disrupted, *T. gondii* cannot survive within host cells. Host immune cells not only recognize *T. gondii*-derived ligands to activate acquired immunity but also destroy the PVs to remove proliferating *T. gondii* and ultimately block parasite expansion in vivo. However, T. gondii secretes various virulence factors into host cells to inhibit host acquired and innate immune responses **(Sasai and Yamamoto, 2019).**

2-9 Immune response in toxoplasmosis

T. gondii is one of the most successful parasites in the world which can produce asymptomatic life-long chronic infection inside the host. The immune response to *Toxoplasma* infection is unique, complicated and compartmented. It has the ability to acquire a balance between the immune strategies for evasion and the immune response of the host, with the aim of not only maximizing the parasite proliferation, but also at minimizing host immunopathology (**Nissapatorn and Khairul, 2004**). A balanced interaction between neutrophils, enterocytes, dendritic cells, and macrophages create the immune response to *T. gondii*. These interactions occur through a complicated group of molecular signaling pathways that bring about regulation and activation of cytokine responses as well as production of effecter molecules.

The high level of genetic heterogeneity background may cause individual variations in immune response. In addition, *Toxoplasma* infection has the ability to spread in all the organs and tissues. Each tissue

compartment possesses its own specific immune response, especially in the placenta and in the central nervous system. An additional level of complexity occurred due to the possibility of reactivation of infection which may vary with strain virulence (Waree, 2008).

2-10: Laboratory diagnosis of *Toxoplasma gondii*

2-10-1: Direct methods :

- Isolation of the parasite or bioassay
- Cellular culture
- Histology

2-10-2: Immunologic diagnostic methods for detection of *Toxoplasma gondii* :

- Sabin and Feldman test
- Hemagglutination
- Modified Agglutination Test (MAT)
- Indirect immunofluorescence (IFI)
- Western blot
- Immunocytochemistry and immunohistochemistry

- Recombinant antigens
- Enzyme linked immunosorbent assay (ELISA) : In this system, the antigen or the antibody is absorbed to an insoluble solid phase (micro polystyrene plates). There are several variants of ELISA: direct, indirect, capture and competitive. All of them allow the determination of antigens in biological fluids except the indirect method that only detects antibodies. Interpretation of the test: If ELISA is qualitative, a development of color is observed in seropositive samples **(Liu *et al*., 2015).**
- Avidity test :This assay measures the avidity of the binding of specific antibodies to *T. gondii* antigen. The proteins present in the serum are denatured with a solution of urea. The avidity can be variable during the course of infection. In early stages of infection, the values of avidity are low and are increasing with the course of infection **(Galvan-Ramirez *et al.*, 2017).**

2-10-3: Molecular techniques

2-10-3-1: Molecular diagnosis by the polymerase chain reaction (PCR)

The Polymerase Chain Reaction (PCR) consists in making a repetitive replication *in vitro* of specific DNA sequences. Amplifying or copying several times a fragment of DNA, the analytical sensibility of the test increases proportionally. The amplified product is analyzed by electrophoresis in agarose or polyacrylamide gel. This technique is useful in the detection of the parasite in serum samples, peripheral blood mono-nuclear cells (PBMC), urine, placenta, amniotic and spinal brain liquid. The sensibility and specificity of PCR depends on the technique used in

the extraction of DNA, the primers and the parameters of the amplification reaction **(Yamagish and Suzuki, 2015).**

2-10-3-2: PCR in real time

Unlike the conventional PCR, the real-time PCR using fluorescence probes measures the product of amplification in each cycle and can be quantified with the use of known concentration standards Real-time PCR has been successfully used to detect the T. gondii DNA in human blood, cerebrospinal fluid, aqueous humour, amniotic liquid and other samples **(Zainodini et al., 2014).**

2-10-3-5:Polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLPs)

The PCR-RFLP is based on the capacity of restriction endonucleases that recognize polymorphisms of only one nucleotide (SNPs). Polymerase chain reaction (PCR) products treated with restriction enzymes to generate polymorphic DNA fragments of variable length, that are visualized after electrophoresis in agarose or polyacrylamide gels with a band pattern **(Galvan-Ramirez *et al.*, 2017).** The tandem repetitions in *T. gondii* are often simple, with only repetitions of two nucleotides, which occur from 2 to 20 times **(Costa *et al.*, 2012).**

2-11:Interleukin-8

The name of Interleukin-8 (IL-8) is emblematic for the “birth of chemokine field **(Moore *et al.*,2019).**IL-8 is contributive to normal and temporary pathological processes. IL-8 induces attraction of specific leukocyte populations during the peri-implantation phase of the embryo **(Caballero-Campo *et al.*,2002).**In infections and injuries, IL-8, released by

macrophages, epithelial or endothelial cells, induces attraction of neutrophils to the affected sites. In response to this event, the clearance of pathogens, the activation of angiogenic response, and the formation of new blood vessels occur **(Brinkmann *et al.*,2002).**

The role of IL-8 is continuously expanding. From the intensely acknowledged pro inflammatory chemokine investigated mainly in chronic inflammatory diseases **(Russo *et al.*, 2014)**and target for possible anti-inflammatory therapeutic strategies **(Gonzalez-Aparicio and Alfaro.,2019)**. to that of main tumor regulator (**Debnath and Neamati.,2017)** and most recently, biomarker for SARS-COV2 viral infections**(Gao *et al.*,2021).**

Chemokines (CKs), also known as chemotactic cytokines (CyKs) (Greek kinos-movement),are small polypeptides of 8-10 kDa, capable to induce chemotaxis in their neighboring cells(**Payne and Cornelius.,2002)**. There are 48 CKs (referred to also as ligands-L/CKLs) grouped in four major subclasses according to structural particularities, XCL, CCL, CXCL, and CX3CL. Their receptors (CKRs) named accordingly are associated with G-proteins and bind several ligands from the same family. The CKs in tandem with their CKRs are involved in tumor pathogenesis through numerous complex processes **(Balkwill,2004;Marcuzzi *et al.*,2019).**

2-12:Interleukin-17A

IL-17A is a cytokine that was recently shown to primarily produced by aCD4+ T cell subset, T helper 17 (Th17) cells **(Wright *et al.*,2007).** The Interleukin-17a gene, originally called the cytotoxicT lymphocyteassociated antigen 8 (Ctla8) gene, was first cloned from a murine cytotoxic T lymphocyte (CTL) hybridoma cDNA library. Murine IL-17A is a 21 kDa glycoprotein containing 147 amino acid residuesthat

shares 63% amino acid identity with human IL-17A(155 amino acids), and both mouse and human IL-17A are secreted as disulfide-linked homodimers. Five additional structurally related cytokines were recently identified: IL-17B,IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F to formthe IL-17 family **(kolls and Linden,2004,Weaver *etal*.,2007).** and each additional member of the IL-17 family shares 16%–50% amino acid identity with IL-17A. IL-17A and IL-17F share the highest amino acid sequence identity (50%), whereas IL-17E is the most divergent,with 16% identity to IL-17A. Amino acid similarity is higher in the C terminus and in five spatially conserved cysteine residues, fourof which form a cystine knot fold that differs from the canonical cystine knot that is observed in transforming growth factor (TGF)-b, bone morphogenic protein, and nerve growth factor superfamilies as a result of the absence of two cysteine residues **(Gerhardt *et a*l., 2009; Hymowitz *et al*., 2001)**. The sequences of IL-17B, IL-17C, and IL-17E differ substantially from those of IL-17A and IL-17F in the N terminus, with longer extensions for the former three proteins. Furthermore, IL-17B is secreted as a noncovalent dimer, suggesting that IL-17B, IL-17C, and IL-17E may form a distinct subclass **(Gerhardt *et al*., 2009;Hymowitz *et al*., 2001).**The Il17f gene is closely located to the Il17a gene in both humans and mice (mouse chromosome 1,human chromosome 6, respectively), whereas genes for other members are located on different chromosomes.The IL-17 receptor (IL-17R) family includes five members(IL-17RA to IL-17RE), which contain such conserved structural characteristics as extracellular fibronectin III-like domains and cytoplasmic similar expression to fibroblast growth factor,IL-17R, and Toll-IL-1R family (SEFIR) domains **(reviewed inGaffen, 2009**). Functional receptors for IL-17 family cytokines are thought to consist of homodimers or heterodimers.For example, the heterodimer of IL-17RA

and IL-17RC is a receptor for homodimers and heterodimers of IL-17A and IL-17F,whereas the heterodimer consisting of IL-17RA and IL-17RBserves as a receptor for IL-17E. Both IL-17B and IL-17E bindto IL-17RB. IL-17C was recently reported to bind to IL-17RE and activate NF-kB. IL-17RD, also called Sef, is preferentially expressed in endothelial, epithelial, and smooth muscle cells, but not leukocytes. The ligands for this receptor, however, have yet to be identified**(Gerhardt *et al.*, 2009)** .

Chapter Three

Materials and Methods

Chapter Three

3- 1:Materials and Methods

3-1-1: The Equipment's and Tools

Table (3-1): The Equipment's and Tools used in the Current Study.

No.	Instruments	Company	Origin
1	Buchner funnel	LAB.	Germany
2	Digital camera	Sony	Japan
3	EDTA tube	Argelik	Jordan
4	Electrophoresis unit	Labnet	Taiwan
5	Eppendorf tubes	Afco	Jordan
6	Filter paper	Broche	Turkey
7	Flask	Marienfeld	Germany
8	Gel tubes	Afco	Jordan
9	Incubator	Memmert	Germany
10	Macro centrifuge	Hitachi	Germany
11	Micro centrifuge	Hitachi	Germany
12	Micropipette	Eppendorf	Germany
13	Microwave	Argos	Germany
14	Nanodrop2000	Thermo	USA

15	Rack	Afco	Jordan
16	Refrigerator	Hitachi	Japan
17	Sample cup	Roche	Germany
18	Sensitive balance	Precisa	Switzerland
19	Syringes	Medo	U.A.E
20	Thermo cycle (PCR)	Labnate	USA
21	Tips	Sterile	UK
22	U.V Light	Quantum	France
23	Vidas	Biomerieux	France
24	Vortex	Labinco	Japan
25	Water bath	Memmert	Germany

3-2: Biological and Chemical Materials

Table (3-2): Biological Materials and Chemical Material used in the Current Study.

Parameters	Company	Origin
Agarose	Biobasic	Canada
Distilled water	Iraq	Iraq
DNA Ladder (DNA marker 100bp)	Promega	USA

Ethanol (96~100%)	Flukachemika	India
Ethidium bromide	Biobasic	Canada
Green master mix	Promega	USA
Humen *Toxoplasma* IgG	Biomerieux	France
Humen *Toxoplasma* IgM	Biomerieux	France
Manganese dioxide	CDH	China
Nuclease free water	Promega	USA
Primers	Promega	USA
Proteinase K	Biobasic	Canada
Tris Borate-EDTA–Buffer solution (TBE)10x	Biobasic	Canada
Tris-EDTA Buffer solution (TE) or Elution buffer	Favorgen	Taiwan
Wash 1 buffer	Favorgen	Taiwan
Wash 2 buffer	Favorgen	Taiwan

3-3: Experimental Design of the Study

The overall procedures of the study summarized in Figure (3-1).

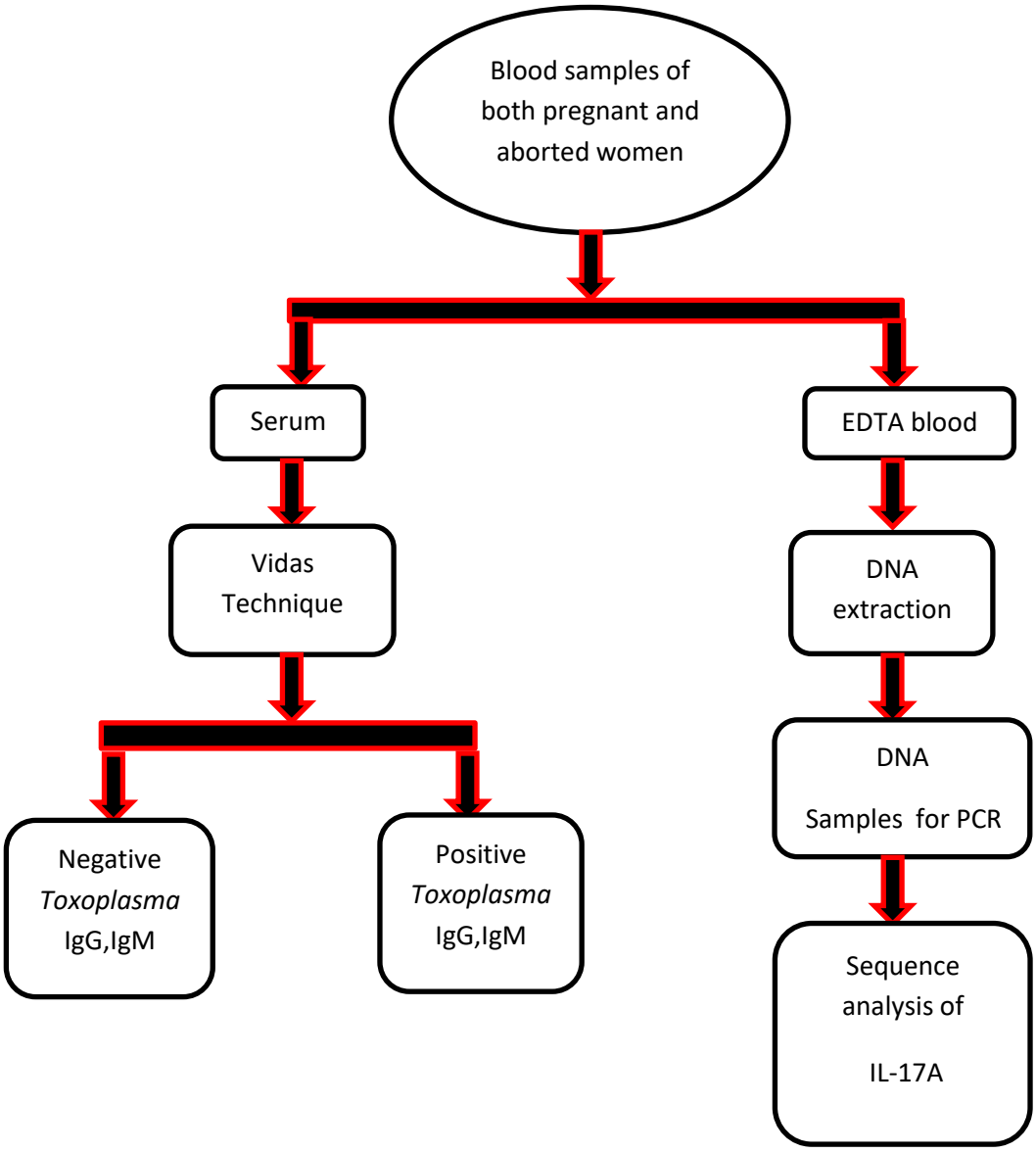


Figure (3-1): Experiment design of the present study.

3-4: Methods

3-4-1: Samples Collection

A total of 96 blood samples from pregnant and aborted women chosen at random from Baghdad Hospital for pregnant and aborted women in Baghdad province,Their age groups ranged from 18 to 45 years and more during the period from October till December 2022. Five milliliters of blood were drawn from each pregnant and aborted women Tow milliliters Blood specimens were saved under -20°C in frozen stat until uses, and the samples were divided into two parts as the following :-

1- Filling an EDTA tube with two mL of blood, for the purpose of a molecular study for IL-8 and IL-17A.

2- To get serum, place three mL of blood in a gel tube, for serological tests (Jones *et al*., 2014).

3-4-2: Blood Samples Collection

Before obtaining a blood sample, all pregnant and aborted women must provide some information as aquestionnaire sheet. Appendix (1).

1. A sterile syringe was used to take blood from the antecubital vein.
2. It took five milliliters of blood for pregnant and aborted women.
3. For serological testing and a molecular study for IL-8 and IL-17A, three milliliters of blood were placed in a gel tube and allowed to clot for 20 minutes at room temperature before being centrifuged at 3000 rpm for five minutes to extract serum,two milliliters of blood were placed in EDTA tube for the purpose of a molecular study for IL-8 and IL-17A.
4. To avoid melting of the samples and repeating the freeze that will affect the quality of the results, the sera obtained were separated into multiple pieces for various serological testing.

5. All sera were kept frozen at -20°C until they were tested against *T. gondii* antibodies (Lima-Oliveira *et al*., 2015).

3-4-3: Sterilization Methods

Sterilization of the tools by autoclave at 121°C for and pressure 1.5 bar for 15 min.

3-5: Immunological assay

3-5-1: VIDAS analyzer to detection the IgG and IgM

This assay was performed by using two kits, one for detection of *Toxoplasma* IgG antibodies, and the other for detection of *Toxoplasma* IgM specific antibodies .

Picture(3-1): The vidas technique.

3-5-2: Detection of Anti-*T. gondii* Antibody (IgG) by vidas Technique

3-5-2-1:PRINCIPL

The assay principle combines an enzyme immunoassay method by immunocapture with a final fluorescent detection(ELFA).The single-use solid phase receptacle (SPR) serves as the solid phase as well as the pipetting device. Reagents for the assay are ready-to-use and pre-dispensed in the sealed single-use reagent strips.All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR device several times.After a sample dilution step, the IgM are captured by the polyclonal Ab coating the interior of the SPR device. Theanti‑toxoplasma IgM are specifically detected by inactivated toxoplasma antigen (RH Sabin strain), which is itself
revealed by an alkaline phosphatase-labeled murine monoclonal anti-toxoplasma antibody (anti-P30).During the final detection step, the substrate (4-Methylumbelliferyl phosphate) is cycled in and out of the SPR device.The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of antibody in the sample.At the end of the assay, an index is automatically calculated by the instrument in relation to the S1 standard stored in memory. The results can then be printed out.

Table(3-3)Content of the kit :

30 TXM Strips	STR	Ready-to-use
30 TXM Solid Phase Receptacles 1 x 30	SPR	Ready-to-use. Interior of SPR devices coated with anti-human µ chain antibodies (goat).
TXM Standard		Human serum* containing anti-*Toxoplasma* IgM + protein

1 x 2.3 mL (liquid)	S1	stabilizer + 1 g/L sodium azide.
TXM Positive control 1 x 2 mL (liquid)	C1	Human serum* containing anti-*Toxoplasma* IgM + protein stabilizer + 1 g/L sodium azide. MLE data indicate the index: confidence interval ("Control C1 (+) Test Value Range").
TXM Negative control 1 x 2 mL (liquid)	C2	Human serum* negative for anti-*Toxoplasma* IgM + protein stabilizer + 1 g/L sodium azide
Specifications for the factory master data required to calibrate the assay: MLE (Master Lot Entry) barcode printed on the box label.		
1 package insert provided in the kit or downloadable from www.biomerieux.com/techlib.		

3-5-2-2:The SPR device

The interior of the SPR device is coated during production with anti-human μ chain antibodies (goat). Each SPR deviceis identified by the "TXM" code. Only remove the required number of SPR devices from the pouch and carefully reseal the pouch after opening.

3-5-2-3:The Reagent Strip

The strip consists of 10 wells covered with a labeled foil seal. The label comprises a barcode which mainly indicates theassay code, kit lot number, and expiration date. The foil of the first well is perforated to facilitate the introduction of thesample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The wells in the centersection of the strip contain the various reagents required for the assay.

Table(3-4)Description of the TXM strip:

Well	Reagents
1	Sample well
2	Serum diluent: TRIS buffer (50 mmol/L) pH 7.4 + protein and chemical stabilizers + 0.9 g/L sodium azide (300 µL).
3	Pre-wash buffer: TRIS (50 mmol/L) pH 7.4 + protein and chemical stabilizers + 0.9 g/L sodium azide (600 µL).
4-5-7-8	Wash buffer: TRIS (50 mmol/L) pH 7.4 + protein and chemical stabilizers + 0.9 g/L sodium azide (600 µL).
6	Conjugate: alkaline phosphatase-labeled immune complex (*toxoplasma* antigen RH Sabin strain grown in mice(12) - mouse monoclonal anti-P30 antibodies) + 0.9 g/L sodium azide + 0.02% gentamicin (400 µL).
9	Empty well.
10	Reading cuvette with substrate: 4-Methyl-umbelliferyl phosphate (0.6 mmol/L) + diethanolamine (DEA*) (0.62 mol/L or 6.6%, pH 9.2) + 1 g/L sodium azide (300 µL).

3-5-2-4:Samples

Specimen type and collection

Serum. It is recommended that each laboratory checks the compatibility of collection tubes used.The use of cord blood or neonatal sera has not been validated. For these specimens, we recommend the use of the Toxo‑ISAGA technique reference 75361.

Sample-related interference

It is recommended not to use hemolyzed, lipemic, icteric samples, and, if possible, to collect a new sample.Refer to the section.

Performance – Study of drugs and other potentially interfering substances for the compounds tested.

Sample stability

Samples can be stored at +2°C/+8°C in stoppered tubes for up to 7 days. If longer storage is required, freeze the sera at-19°C/-31°C.Avoid successive freezing and thawing.

Instructions for use

For complete instructions, see the Instrument User Manual.

Reading MLE data When opening a new lot of reagents With the external instrument barcode reader, scan the MLE data on the box label before performing the test.

If this operation is not performed **before initiating the tests**, the instrument will not be able to print results.

Note: The master lot data need only be entered once for each lot.

It is possible to enter MLE data manually or automatically depending on the instrument (refer to the User Manual).

Calibration

Calibration, using the standard provided in the kit, must be performed each time a new lot of reagents is opened, after the MLE data have been entered, and then every 14 days. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf life of the kit.

The standard, identified by S1, must be tested in duplicate.

The standard value must be within the set RFV (Relative Fluorescence Value) range indicated in the MLE data. If this is not the case, recalibrate.

3-5-2-5:Procedure

1. Only remove the required reagents from the refrigerator and allow them to come to room temperature for at least 30 minutes.

2. Use one "TXM" strip and one "TXM" SPR device for each sample, control or standard to be tested. Make sure the SPR pouch has been carefully resealed after the required SPR devices have been removed.

3. The test is identified by the "TXM" code on the instrument. The standard must be identified by "S1" and tested in duplicate. If the positive control is to be tested, it should be identified by "C1". If the negative control needs to be tested, it should be identified by "C2".

4. If necessary, clarify samples by centrifugation.

5. Mix the standard, controls and samples using a vortex-type mixer (for serum separated from the pellet).

6. For this test, the standard, control, and sample test portion is 100 µL.

7. Insert the "TXM" SPR devices and "TXM" strips into the instrument. Check to make sure the color labels with the assay code on the SPR devices and the Reagent Strips match.

8. Initiate the assay as directed in the User Manual. All the assay steps are performed automatically by the instrument.

9. Close the vials and return them to +2°C/+8°C after pipetting.

10. The assay will be completed within approximately 40 minutes. After the assay is completed, remove the SPR devices and strips from the instrument.

11. Dispose of the used SPR devices and strips into an appropriate recipient.

3-6: Molecular assay

3-6-1: The extraction of DNA from blood

1. Collected frozen human blood in an anticoagulant-treat collection tube and waited at room temperature until it thawed and gently mixed.
2. Transferred 300µl of blood to a microcentrifuge tube for each sample .
3. Added 30ul of Protinase K for each sample and mixed gently .
4. Incubated for 20 minutes at 56°C temperature .
5. A 200µl FABG Buffer added and vortex combined .
6 .Incubated at 70°C for 15 minutes. Invert the sample every 3 ~ 5 minutes, during incubation .
7. The sample was supplemented with 200µl ethanol (96 ~ 100 per cent). Mix vigorously for 10 seconds with overtaxing 30 min.
8- Pick up spin column tubes in their collection tubes
9. Transverse all lysate into spin column of samples for each.
10. Spin the micro centrifuge for 1minute.Dischaed the filtrate lysate and replace the collection tubes.
11. Washed FABG Column with 400µl W1 Buffer (ethanol added) by centrifuge for 30 seconds.
12. Washed FABG Column with 600µl Wash Buffer (ethanol added) by centrifuge for 30 seconds.
13. Centrifuged for an additional 3 min to dry the column.
14. Putted the FABG Column tubes to a new 1.5ml microcentrifuge tubes for each.
15. Added 100µl of Preheated Elution Buffer or TE to the membrane center of FABG Column. Stand FAGB Column for 3~5 min or until the buffer is absorbed by the membrane.
16. Centrifuged for 30 seconds to elute the pure DNA .

17. Stored the DNA fragment at 4ºC or -20ºC.(Al-Jubory and Imran 2020).

3-6-2:Estimation the DNA Concentration and Purity

The DNA concentration of samples was estimated by using the Nanodrop by putting 1 µl of the extracted DNA in the machine to detect concentration in ng/µl and the purity detected by noticing the ratio of Optical Density (OD) 260/280 nm to detect the contamination of samples with protein. The accepted 260/280 ration for purifying DNA was between 1.7-1.9 (Sambrook and Russell, 2001).

3-6-3: Primers

The primer IL-8 pair was used in the current study are listed in Table (3-5).

Gene	Primer sequence	Tm	Product size	Reference
IL8 -251T fi A (rs4073).	Forward inner primer (T allele): GTTATCTAGAAATAAAAAAGCATACAA	58C	169 bp (T allele)	Melanie *et al.*,2006
	Reverse inner primer (A allele): CTCATCTTTTCATTATGTCAGAG		228 bp (A allele)	
	Forward outer primer CATGATAGCATCTGTAATTAACTG		349 bp (two outer primers)	
	Reverse outer primer CACAATTTGGTGAATTATCAAA			

The primer IL-17A pair was used in the current study are listed in Table (3-6).

Primers	DNA equences s	Size	Tn	Gene	References
F17A	AATCAAGGTACATGACACCAG	694bp	56	IL17A	Designed by Author
R17A	TTAGCCCCAATATAGCTATCTT			IL17A	

3-6-4: Dissolving of Primers

Nuclease free water was used to dissolve primer pair utilized in this study (Promega). The primer stock tube would be prepared first, and then the working solution would be created from the primer stock tube.

Nuclease free water was added according to the primer manufacturer's instructions to achieve a primer stock solution concentration of 100 picomol/microliter. The working solution prepared from stock by dilution 10µl primer stock solution with 90 microliter of nuclease free water to get 10 picomol/microliter (work solution).

3-6-5: Prepared the Reaction Mixture

Amplification of DNA was carried out in a final volume of 25 µl reaction mixture for detection of IL-8 and IL-17A polymorphism as mentioned in Table (3-4) as mentioned adding (1µl) from each diluted primer.

Table(3-7):The final volume of 25µl PCR reaction component

No.	Component	Quantity
1	Mater mix	13
2	10 µM forward Primer	1.2
3	10 µM reverse Primer	1.2
4	Template DNA	1.6
5	Nuclease-free water	8
Approximately final volume		25

3-9-6: Thermal Cycling Conditions

The PCR reaction is shown in Table (3-8).

Table (3-8): Thermal cycling conditions for specific primers for IL-17A and IL-8 gene under interest.

Step Type	Temperature °C	Time	Cycling
Initial Denaturation	93	3 min.	1
Denaturation	93	30 Sec.	32
Annealing	47	30 Sec.	
Extension	72	30 Sec.	
Final Extension	72	3 min.	1
Hold	4	∞	

3-7: Validity of the IL17A genotype

The genotypes of IL17A SNP (NCBI SNP CLUSTER ID:rs2275913) were analyzed by polymerase chain reaction (PCR) and sequencing investigation according to the conventions depicted before

based on Govatati *et al*. (2012) method. PCR were performed in a complete volume of 25 µl containing 50 ng genomic DNA, 1µl of primers, 12.5 µl master mix in Table (3-6). The PCR result of 986 bp was electrophoresis by 1.5% agarose gel staining with ethidium bromide and afterward sequenced in Macrogen Lab. (Korea).

3-8: Detection of Amplified Products by Agarose Gel Electrophoresis

The amplified products of PCR were separated by electrophoresis on a 1.5% agarose gel stained with ethidium bromide. Agarose gel was prepared by dissolving 1.5 g of agarose powder in 100 ml of 1X TBE buffer in flask then placed in microwave for 2 minutes, and waiting for cool to 50°C and adding about 0.5µl of ethidium bromide stain concentration (0.5 mg/ml) (Sambrook and Russell, 2001).

First the 4µl of DNA ladder (100-10kbp) was put. The electric voltage 75 volt at 45 minute (Sambrook and Russell, 2001). Gel documentation system was used to capture the pictures of the gel and observation of DNA bands under UV light.

3-9: Interpretation of Sequencing Data

About 18-20 µl of PCR products of all genes under interest in this study samples were sent to the macrogen company in South Korea to performed the sequence of DNA for detection SNPs under interest. Through 15 days the data of sequencing received by email in three formulas; pdf file, text document sheet and AB1 file which requires sequencing reading program by Geneious prime purchased version. In addition, the NCBI data tools were used for alignment the gene sequence by BLAST tool of NCBI.

3-10:Statistical Analysis

All statistical calculation was performed by the use of SPSS software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp. USA) and Microsoft Excel (2010, Microsoft Corp. USA). A $p < 0.05$ was considered statistically significant. Chi-square test to assess the categorially association variables and genetic association, according to (Kang & Shin, 2004).Allele frequencies of genes were calculated by direct gene counting methods, while a significant departure from Hardy-Weinberg (H-W) equilibrium was estimated using H-W calculator for two alleles.

Chapter Four
Results and Discussion

Chapter Four

4- Results and Discussion

4-1: Epidemiological Criteria

This study included (96) pregnant and aborted women. The results showed that (20) of them were infected with toxoplasmosis (14) sample for(78)sample to pregnant and (6) sample for(18)sample to aborted women , while (64) of them were not infected for pregnant and(12) sample not infected for aborted women. The purpose of this study was to determine the effect of toxoplasmosis on IL-8 and IL-17A, among the infected pregnant and aborted women and not infected throughout measuring toxoplasma IgG and IgM levels in relation with these parameters.

Table(4-1): Percentage of infection of toxoplasmosis in pregnant and aborted women by using the vidas technique.

Women Status	Examined No.	Positive		Negative		Positive		Negative		P.value	
		IgG	%	IgG	%	IgM	%	IgM	%	IgG	IgM
Pregnant	78	14	17.99	64	82.05	0	0	78	100	0.147	0.72
Aborted	18	6	33.3	12	66.6	0	0	18	100		
Tatal No.	96	20	20.83	76	79.16	0	0	96	100		
Calculatedx^2 / Tabulatedx^2	IgG	2.09 / 3.84									
Calculatedx^2 / Tabulatedx^2	IgM	0.12 / 3.84									

Results in table (4-1) showed that of the total (96) pregnant and aborted women, 14(17.99%)for pregnant and 6(33,3%) for aborted had positive IgG antibodies to toxoplasmosis, while 64 (82.5%) for pregnant and 12(6,66%)had negative IgG antibodies to toxoplasmosis. The results of a positive sample by using VIDAS technique for IgG and antibodies (IgG&IgM) among aborted and pregnant women that it's indicated non- significant difference of infection by *T.gondii* according to women status. In the aborted women that higher than compared with pregnant women for IgG, The study agreement with the study of the Al-Dulaimy (2015) in IgG was 41.81% in aborted and 40.0% in pregnant women, also in other study Al-Dahmoshi *et al*. (2013) in the repeated abortion women who found the percentage for IgG, (IgG&IgM) were 26.5%, 11.8%, respectively. In agreement with study in aborted women, also in aborted women the IgG rates disagreement with Yacoob *et al*. (2006) who they found were 52.1% in Basrah also in pregnant the rates of IgG approximately disagreement with study of Kareem (2008) who found the percentage of infection among pregnant women were 31.04%, also disagreement with study Al-Mosawi (2012) who found the percentage was 43.7%. Whereas for the IgM disagreement with study Al-Dulaimy (2015) who found 0.0%, 0.0%, in aborted and pregnant women respectively.

Table(4-2) :Percentage of infection toxoplasmosis in the pregnant and aborted women by using the vidas technique according to the Age(year).

Age groups	Examined No.	Positive		Negative		Positive		Negative		P.value	
		IgG	%	IgG	%	IgM	%	IgM	%		
18-22	16	3	18.75	13	81.25	0	0	16	100	IgG	IgM
23-27	22	4	81.81	18	81.81	0	0	22	100	0.909	0.630
28-32	24	6	25	18	75	0	0	42	100		
33-37	16	3	18.75	13	81.22	0	0	16	100		
38-42	15	4	26.66	11	73.33	0	0	15	100		
43 and more	3	0	0	3	100	0	0	3	100		
Total No	96	20	20.83	76	79.16	0	0	96	100		

Calculatedx^2	IgG	1.52
Tabulatedx^2		12.59
Calculatedx^2	IgM	3.5
Tabulatedx^2		12.59

Results in table (4-2) showed that of the total (96) pregnant and aborted women, according to the division of the study samples into six age groups, the results show the percentage is(18.5%) in the age groups (18-22) years in pregnant and aborted women patients with toxoplasmosis, the percentage

is(18.18%) in age groups(23-27) years in patients with toxoplasmosis, while the percentage is (25%) in the age groups (28-32) years in patients with toxoplasmosis. The highest percentage is (18.75%)in the age groups (33-37) years in patients with toxoplasmosis, while the highest percentage is (26.66%) in the age groups (38-42) years and (0%) in the age groups (42 and more) years in pregnant and aborted women patients had positive IgG antibodies with toxoplasmosis.0 (0%) had positive IgM antibodies to toxoplasmosis in all age groups. And this table is non-significant. By using VIDAS test for IgG in pregnant and aborted women where focused in (18-22) years as (18.18%), agreed with Al-Mosawi (2012) which showed dissimilar findings with *Toxoplasma* seroprevalence 25-29 years for IgG was 66.6% . also agreement with Abdulla *et al*.(2015) who found in age group(31-40)years with IgG was 35% .

VIDAS-IgM was recorded variable results in the pregnant and aborted women 0%, the present study was disagreement with the study of Saleh (2011) which proved that age group 19-29 years was 10.8% as well as with study of Mahmood *et al*. (2013*)* showed similar findings with *Toxoplasma* was (18-25) years was 30% with IgM and study of Al-Saadii (2013) in which had noticed (18-25) was 30% also disagreement with study of Al-Jumaili (2015) who found in age group(22-26)years with IgM was 4.6%.

VIDAS-IgG where agreement with Al-Mosawi (2012) who found in age group(20-24) years with (IgG) was 49.2%.

While IgG in pregnant and aborted women disagreed with study of Saleh (2011) which was 30% for age group (40-49) years and study of Al- Maamuri (2014) which was 85.39% for age group (41-50) years.

Table(4-3) :Percentage of infection toxoplasmosis in the pregnant and aborted women by using the vidas technique according to the residence area.

Residence Area	Examined No.	Positive		Negative		Positive		Negative		P.value	
		IgG	%	IgG	%	IgM	%	IgM	%		
Urban	45	14	31.11	31	68.88	0	0	45	100	IgG	IgM
Rural	51	6	11.76	45	88.23	0	0	51	100	0.0198	0.286
Tatal No.	96	20	20.83	76	79.16	0	0	96	100		

Calculatedx^2	IgG	5.42
Tabulatedx^2		3.84
Calculatedx^2	IgM	1.13
Tabulatedx^2		3.84

Results in table (4-3) showed that of the total (96) pregnant and aborted women, according to the division of the study samples according to the residence area in to two residence area (Urban and Rural) the results show the percentage is(31.11%) had positive IgG antibodies in the Urban in pregnant and aborted women patients with toxoplasmosis, the percentage is(11.76%) had positive IgG antibodies in the Rural in patients with toxoplasmosis. 0 (0%) had positive IgM antibodies to toxoplasmosis in all samples table(4-3) significant to IgG and is non significant to IgM. The current study agreement with study of Hamza (2006) which revealed that non-significant association between the residency and prevalence of total *Toxoplasma* antibody by ELISA IgM test for

urban area has (32.8%), and for rural area has (40.3%), and also agreement with study of Jassam (2010) which showed that among the positively rate of anti-*Toxoplasma* IgG antibodies selected explanatory variable among schizophrenic group was non-significant among rural (49.4%) compared to urban residence (47.1%). Results were in agreement with pregnant women in Amol, Northern Iran, the toxoplasmosis in urban areas was less than rural area **(Panah *et al.*, 2013)**. The *Toxoplasma* infection among pregnant women in Northeast Iran living in urban and rural areas were 29.1%, 47.5%, respectively **(Babaie *et al.*, 2013)** that agreement with present study. While agreement with study Al-Jubori (2005) regarding the residency of the patients and its relation with seropositive antibodies showed no significant difference between positive antibodies distribution and both urban and rural areas, through which the rate for urban and

rural areas were (33.98%) and (32.08%) respectively, results (IgG) agreement with Al-Mosawi (2012) who found 44.8% urban area and disagreement with results(IgM) were 39.6% rural area. It may be the reason for this disparity between rural area and urban area belong to sample size, or may be have plenty of infection and disease toxoplasmosis belong to eating infected meat contaminated with parasite where there is a close relationship between farm animals and human consumption of meat goats and sheep (Roberts & Janovy, 2000).

Table(4-4) :Percentage of infection toxoplasmosis in the pregnant and aborted women by using the vidas technique according to Cat Presence.

Cat Presence	Examined No.	Positive		Negative		Positive		Negative		P.value	
		IgG	%	IgG	%	IgM	%	IgM	%		
Cat Presence	56	12	21.42	44	78.75	0	0	56	100	IgG	IgM
Cat not Presence	40	8	20	32	80	0	0	40	100	0.865	0.911
Tatal No.	96	20	20.83	76	79.16	0	0	96	100		

Calculatedx^2	IgG	0.28
Tabulatedx^2		3.84
Calculatedx^2	IgM	0.01
Tabulatedx^2		3.84

Results in table (4-4) showed that of the total (96) pregnant and aborted women. According to Cat Presence the division of the study samples according to Cat Presence in to two (Cat Presence and Cat not Presence), the results show the percentage is(21.42%) had positive IgG antibodies to toxoplasmosis in the Cat Presence in pregnant and aborted women patients with toxoplasmosis, the percentage is(20%) had positive IgG antibodies to toxoplasmosis in the Cat not Presence in patients with toxoplasmosis. 0 (0%) had positive IgM antibodies to toxoplasmosis in all samples. This table non-significant. The results agreement with Al-Maamuri (2014) who found the presence of the cats 88.43% have higher than those who do not have cats 59.7%, and disagreement with Ghasemian *et al.*(2007) that were found 6.1% for not keeping animals and 3.3% for those keeping animals. Various studies have also reported a statistical

correlation between toxoplasmosis prevalence and close contact with cats, such as the study of Al-Shikhly (2012) that found no significant differences between students contact with animals in different universities, that revealed high rate of infection between women not in contact with animals, and this not agree with present study.

the present study disagreement with Al-Mosawi (2012) who found high rates infection for those not exist cats in their houses with (IgG&IgM) were 45.7%.

Table(4-5) :Percentage of infection toxoplasmosis in the aborted women by using the vidas technique according to Abortion No.

Abortion No	Examined No	Positive		Negative		Positive		Negative		P.value	
		IgG	%	IgG	%	IgM	%	IgM	%		
One time	6	2	33.33	4	66.66	0	0	6	100	IgG	IgM
Two time	2	1	50	1	50	0	0	2	100	0.860	0.9799
Three or more time	10	3	30	7	70	0	0	10	100		
Tatal No.	18	6	33.33	12	66.66	0	0	18	100		
Calculatedx^2 Tabulatedx^2	IgG	0.3									
		5.991									
Calculatedx^2 Tabulatedx^2	IgM	0.0404									
		5.991									

Results in table (4-5) showed that of the total (18) aborted women, According to abortion No the division of the study samples according to abortion No in to three groups (one time ,two time, three or more time),the results show the percentage is(33.33%) had positive IgG antibodies to toxoplasmosis in one time abortion in aborted women patients with toxoplasmosis, the percentage is(50%) had positive IgG antibodies to toxoplasmosis in the two time abortion in patients with toxoplasmosis and, the percentage is(30%) had positive IgG antibodies to toxoplasmosis in the three time abortion in patients with toxoplasmosis. (0%) had positive IgM antibodies to toxoplasmosis in all samples. This table is non-significant. The results agreement with study Al-Mosawi (2012) that found IgG, are 35%, respectively,

and disagreement with the study of Al-Kalaby (2008) in Al-Najaf province found the highest ratio for IgG, IgM, are 55.7%, 37.7%. respectively, in the cases one aborted occur, also agreement with study Mohammad *et al*.(2013) who found IgG was 58.83%, respectively, in two times number of abortion and (IgG)were 44.44% in one times number of abortion.

Perhaps the explanation of these results is a reactivation of the parasite in the incidence of latent or chronic, with turn back parasite in the tissue due to hormones and reduced immunity during pregnancy and liberated the parasite infects the placenta and cause inflammation, preventing the arrival of food and oxygen to the fetus and cause a miscarriage and fetal death or transmitted the parasite to the fetus and cause distortions congenital **(Dubey, 2007).**

Table(4-6) :Percentage of infection toxoplasmosis in the pregnancy women by using the vidas technique according to Duration of pregnancy.

Duration of pregnancy	Examined No.	Positive		Negative		Positive		Negative		P.value	
		IgG	%	IgG	%	IgM	%	IgM	%		
1-3	11	1	9.09	10	90.90	0	0	11	100	IgG	IgM
4-6	25	5	20	20	80	0	0	25	100	0.707	0.975
7-9	42	8	19.04	34	80.95	0	0	42	100		
Tatal No.	78	14	17.74	64	82.05	0	0	78	100		

Calculatedx^2 Tabulatedx^2	IgG	0.691
		5.991
Calculatedx^2 Tabulatedx^2	IgM	0.048
		5.991

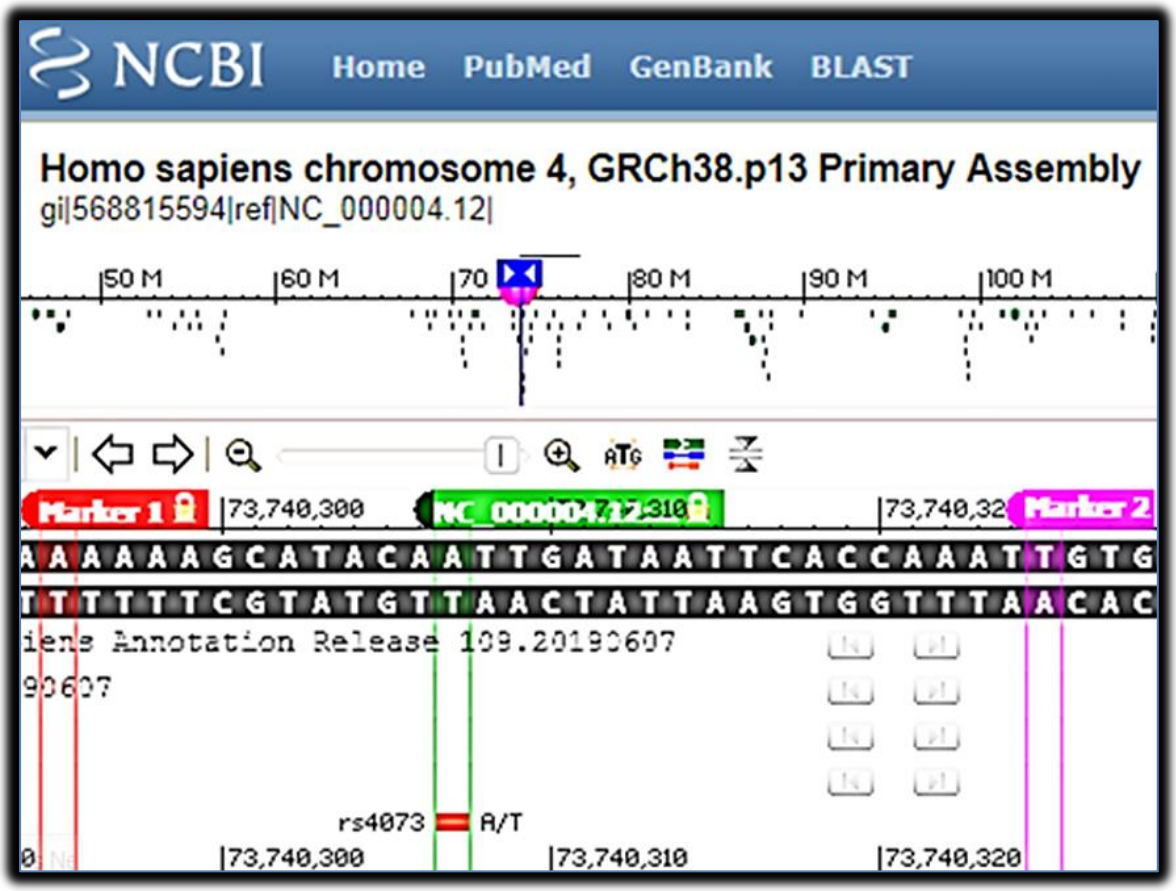
Results in table (4-6) showed that of the total (78)pragnant women, according to duration of pregnancy the division of the study samples according to Duration of pregnancy in to three groups (1-3),(4-6),and(7-9).The results show the percentage is(9.09%) had positive IgG antibodies to toxoplasmosis in(1-3)month in pregnant women patients with toxoplasmosis, the percentage is(20%) had positive IgG antibodies to toxoplasmosis in (4-6)month in pragnany

patients with toxoplasmosis and, the percentage is(19.04%) had positive IgG antibodies to toxoplasmosis in(7-9)month in pragnancy in patients with toxoplasmosis.(0%) had positive IgM antibodies to toxoplasmosis in all samples. Table(4-6) is non-significant. By using VIDAS test The results indicate that the highest percentage of positive cases for IgG, was 20%,19%,04 %,9.09%, respectively, focusing in first trimester(1-3) months (first trimester) . agreement also with study Tkhori *et al*.(2014) who found the rate infection was 13%, but disagreement IgG, with this study found 27.27%, 36.36%, respectively, in the second trimester, the level of IgM disagreement with study Yobi *et al*.(2014) who found the highest percentage in third trimester was 5.8% .High rate of infection in the first period of pregnancy (1-3) months this may be due to the fact that first trimester of the pregnancy is the critical period in which the fetus is not considered a well-established in the womb and it is threatened for abortion whenever the mother is expose to any risky factor such as reactivation of latent infection such as *Toxoplasma* that result from immunosuppressant concomitant with pregnancy which can lead to placental infection and next placental insufficiency, with subsequent embryonic death .This result agreement with other many studies like Kadhim (2007) and Mohammed (2008) .

4-2:Polymorphism of IL-8

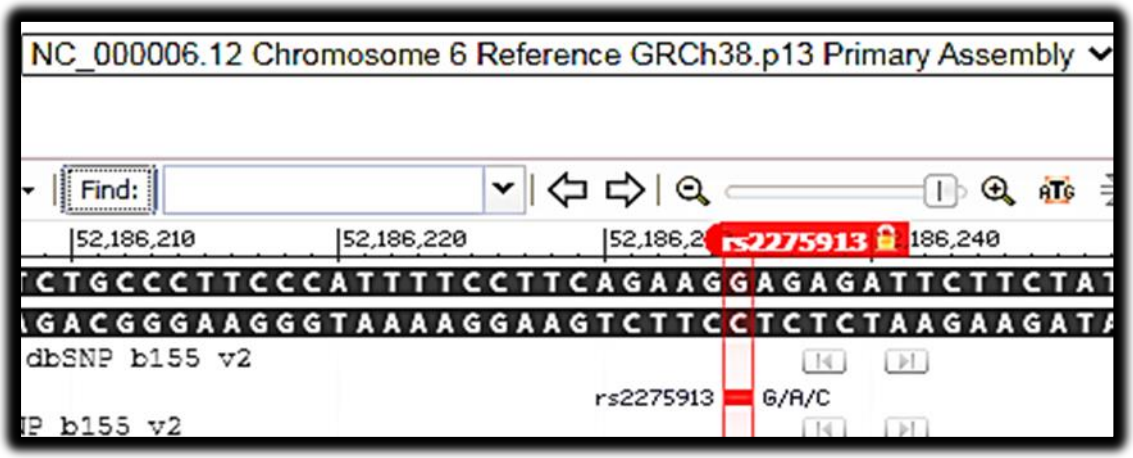
4-2-1:IL-8 gene: Validity SNP rs4073 site:

The rs4073 located on Chromosome 4 Figure(4-1)



4-2-2:Validity SNP rs2275913 site:

The SNP rs2275913 located on chromosome 6 Figure(4-2).



4-3:DNA extraction results for IL-8:

4-3-1:IL-8 gene polymorphism and apportion:

4-3-1-1: IL-8 gene polymorphism of patients group Figure (4-3).

Figure (4-3): Gel profile of IL-8 -251 T>A PCR tetra primer method for 20 patients as case group: 13 samples showed A/T genotype, 4showed TT genotype, and 3 showed AA genotype. Bands: Top Control (349 bp), middle A allele (228 bp), bottom T allele (169 bp). Lane: M: molecular marker size 10kbp (100bp for first step).

4-3-1-2: IL-8 gene polymorphism of patients group Figure (4-4).

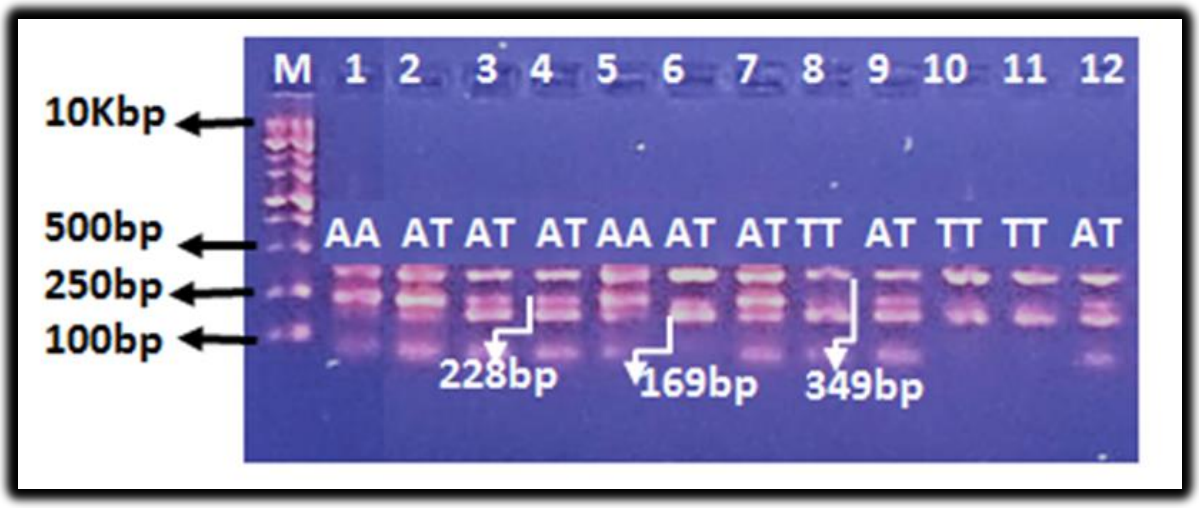


Figure (4-4): Gel profile of IL-8 -251 T>A PCR tetra primer method for control group:7 samples showed A/T genotype, 2 showed TT genotype, and 2 showed AA genotype. Bands: Top Control (349 bp), middle A allele (228 bp), bottom T allele (169 bp). Lane: M: molecular marker (size 10kbp, first step 100bp; = TT homozygous mutant allele; AA homozygous wild allele ; =AT heterozygous mutant allele.

Table (4-7): Genotypes number and allele frequency of SNP rs4073 of IL-8 between patients and control groups ,shown P-value (0.05) and the Odd Ratio values.

Rs4073 A>T	Patients group .N=20		Control N=20	OR(95%CI)	P-value
Genotypes	AA	3(15%)	3(20%)	Reference group	
	AT	14(70%)	12(60%)	**1.55(0.42-5.7)**	0.5
	TT	3(15%)	5(20%)	0.53(0.11-2.5)	0.4
Allele Frequency	A	20(50%)	18(45%)	**1.22(0.51-2.9)**	0.6
	T	20(50%)	22(55%)	0.82 (0.34-1.9)	0.6

Note: If OR value for mutant allele (T) more than one, mean this allele considered as risk allele and caused disease. In this table T allele in heterogeneous AT genotype has OR= **1.55**, it's a risk allele and caused disease.

4-4:DNA extraction results for IL-17A:

The profile gel-electrophoresis 20 blood samples for patients (Figure 4-5), 12 samples for control group Figure (4-6) , huge bright bands of DNA except some samples shown faint bands, this may result due clotting blood samples through processing .

Figure (4-5): Gel-electrophoresis illustration quality of DNA extracted from patients group, 1-20 patient samples, M=molecular marker (size10kbp), First step 100bp.

Figure (4-6): Gel-electrophoresis illustration quality of DNA extracted from control group, 1-12 samples, M=molecular marker (size10kbp), First step 100bp.

4-4-1:Amplification of partial region of IL17A gene:

The Figure 7 shows the targeted region of partial sequence of IL17A was amplified by primer pair covering the amplicon target of patient and control group Figure 7 and 8.

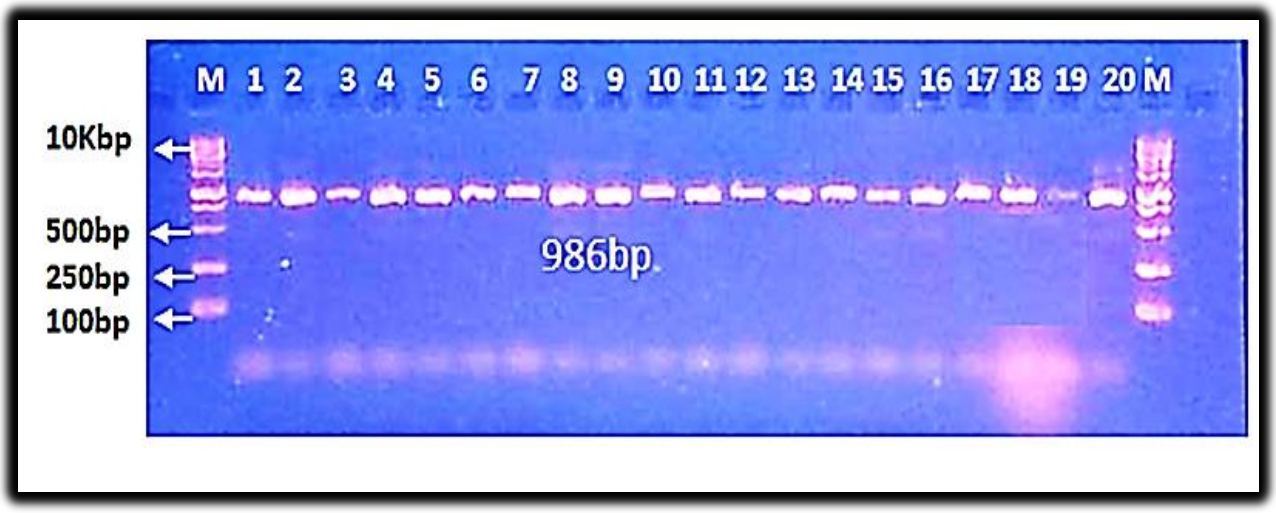


Figure (4-7): Gel electrophoresis 52185415-52186396 as target DNA region of IL17A amplification region with flanking primers, 1-20 patient group, PCR products 986bp, M=molecular marker 100bp for each step.

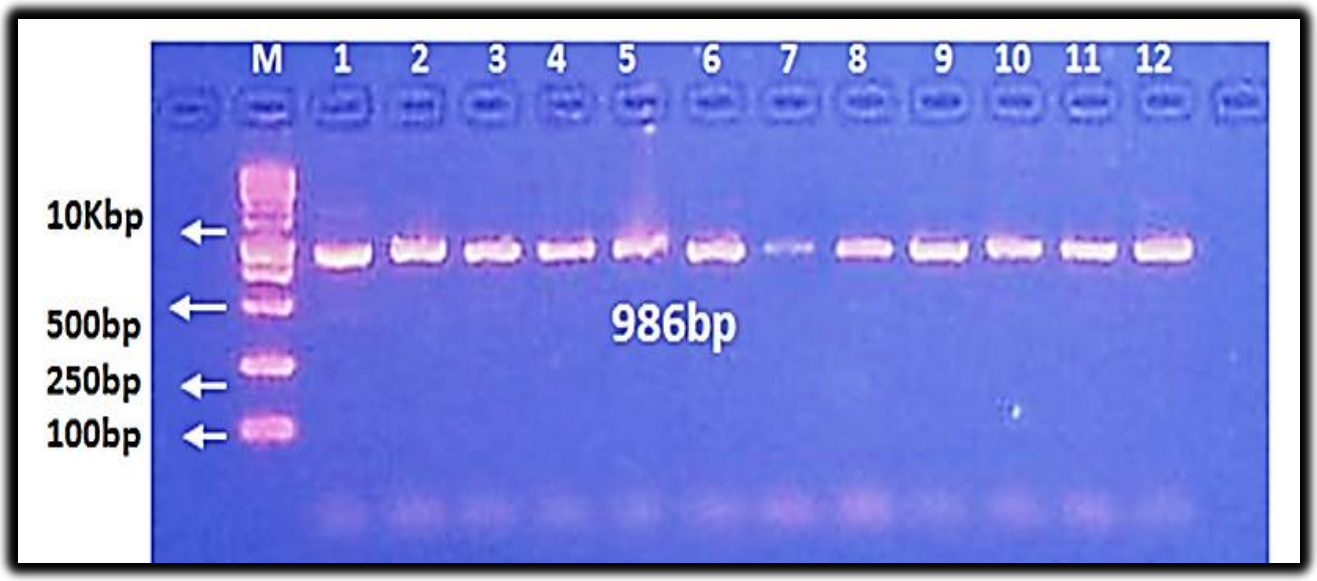


Figure (4-8): Gel electrophoresis 52185415-52186396 as target DNA region of IL17A amplification region with flanking primers, 1-12 control group, PCR products 986bp, M=molecular marker 100bp for each step.

4-5: Sequence results forIL-17A

In order to perform sequence of PCR products, about 18-20µl of PCR product were send to macrogen laboratory (South Korea). After receiving the sequence, the results were subjected for bioinformatics analysis by geneious prime software.

4-6: The multiple alignments of chromatograms T. gondii with aborted and pregnant women.

4-6-1: The multiple alignments of chromatograms of patient cases: The results of targeted region shows one SNP: rs2275913 G>A. Figure (4-9).

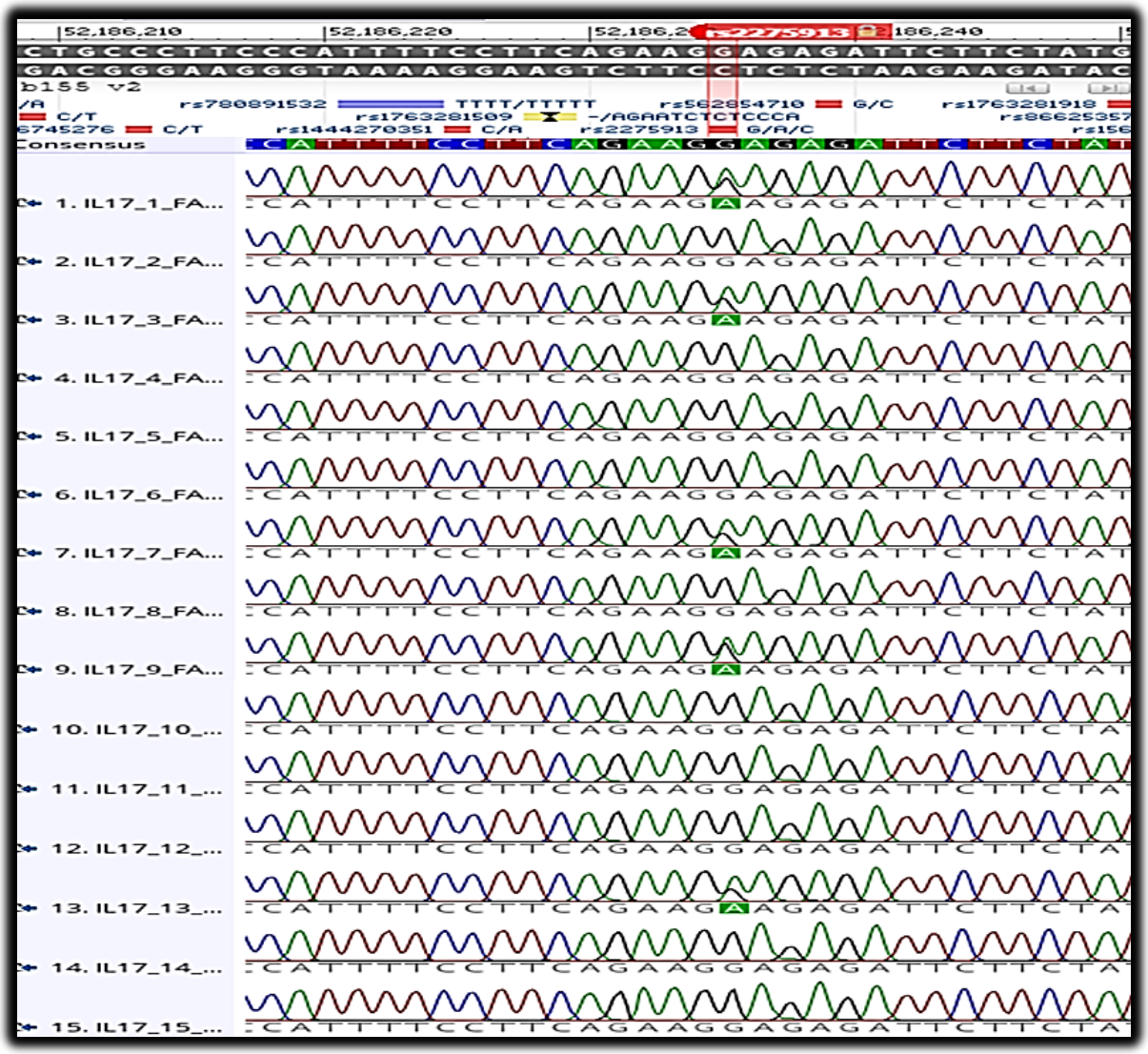


Figure (4-9):Multiple alignment of 10 partial of IL17A sequences of control group, based on Chromatograms peaks, shown the combination of SNP: rs2275913G>A between its position on chromosome 6 and their position in some patient group. The multiple alignments performed by Geneious prime software.

4-6-2:The multiple alignments of chromatograms of control group:

The results of targeted region shows one SNP: rs2275913 G>A. Figure**(4-10)**

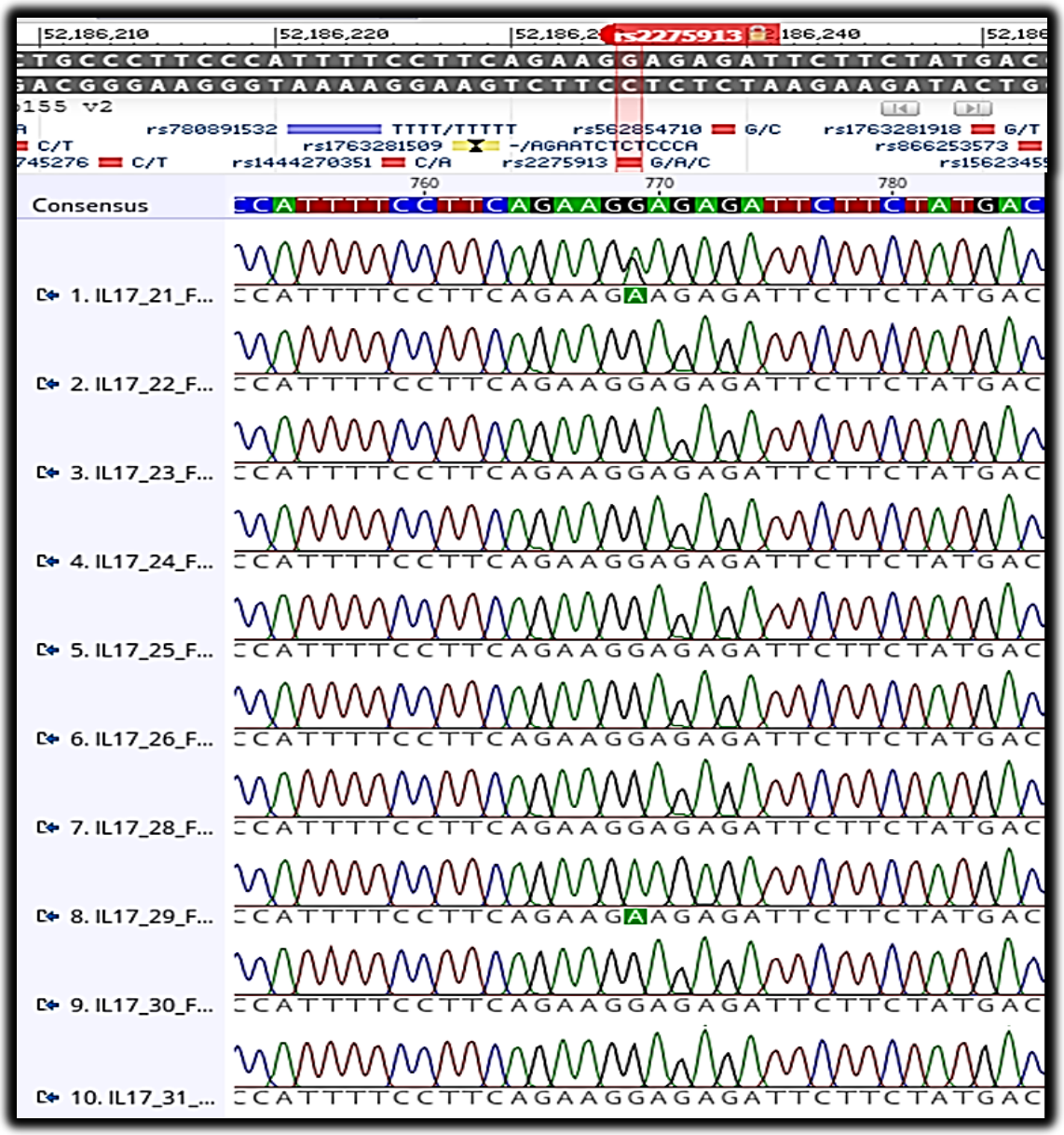


Figure (4-10):Multiple alignment of 10 partial of IL17A sequences of control group, based on Chromatograms peaks, shown the combination of SNP: rs2275913G>A between its position on chromosome 6 and their position in some patient group. The multiple alignments performed by Geneious prime software.

Table (4-8): Genotypes number and allele frequency of SNP rs2275913 of IL17A between patients and control groups ,shown P-value (0.05) and the Odd Ratio values.

Rs2275913 G>A	Patients group .N=20		Control N=20	OR(95%CI)	P-value
Genotypes	GG	14(70%)	16(80%)	Reference group	
	GA	0(0%)	0(0%)	1(0.019-52.03)	1
	AA	6(30%)	4(20%)	**1.7(0.40-7.3)**	3eee3qawsaaaaq
Allele Frequency	G	28(70%)	32(80%)	0.58(0.21-1.63)	0.3
	A	**12(30%)**	8(20. %)	**1.71 (0.61-4.8)**	0.3

As seen in Figures (4-7) and (4-8), the IL-17A SNPs polymorphism the presence of GG genotype of rs2275913 of IL-17A allele is rundamentaly low in control group than patients groups.

This study took up two important status the first one is pregnant and the second is aborted women with toxoplasmosis and polymorphism of IL-8and IL-17A**.**

Toxoplasmosis, caused by *T. gondii*, is a common foodborne opportunistic pathogen that can cause serious illness in immunocompromised patients. In strains from around the world, the pathology and immunological responses associated with the resulting illness have not been thoroughly documented **(Lieberman and Hunter, 2002**). The results of this study are consistent with study results **(Ali *etal* .,2022).**

In this study, the high frequency of allele risk allele A in homozygous genotype (AA) in SNP rs2275913 IL-17A based on the odd ratio was higher in genotype AA, OR=1.7(0.40-7.3) with high significance p-value=0.4, and the allele frequency is higher with allele A=12(30%) in patient group with high value of OR=1.71(0.61-4.8) p-value=0.3, while the allele frequency is low

32(80%) in control group as shown in Table (4-8). The same observation in case (AT and AA) genotypes in SNP of rs4073 IL-8 based on present results, the OR is higher in genotypes AT, OR=1.55(0.42-5.7), P-value=0.5 and for TT the OR= 0.53(0.11-2.5) with high significance P-value=0.4, the allele frequency is higher with allele A=20(50%) in patient group with high value of OR=1.22(0.51-2.), P-value=0.6, while the allele frequency is low 22(55%) in control group as shown in Table (4-7). These causes deviation of the role of IL-17A and IL-8.This deviation was correlated with the function of IL-117A and IL-8.

In general, IL-17A and IL-8 levels are enhanced during the intense and continuous disease, while immunosuppressed patients' IL-17A and IL-8 levels decreased. This anti-inflammatory cytokines can stimulate T helper 17 (Th17) reactions, IL-17A and IL-8 is thought to be a suppressor of Th17 and IL-8, released by macrophages, epithelial or endothelial cells, induces attraction of neutrophils to the affected sites, insusceptible responses (**Wright *et al*.2007, Brinkmann *et al*.,2002**).

as cytokine production is genetically controlled, and IL-17A and IL-8 promoter polymorphisms they have been get engaged to a difference of disorders **(Daher *et al*., 2003; Kamali-Sarvestani *et al*., 2005),** here IL-17A and IL-8 genes polymorphisms have come true in toxoplasmosis and pregnant and aborted women pation and control group. These results demonstrate a role for SNPs cases under interest (**Moore *et al*., 2001**)

The present results are consistent with results of AI-Dahmoshi *et al*. (2013), when he refers to the role of Interleukin-8 (IL-8) and Interleukin-17A(IL-17A) which an important anti-inflammatory cytokines, the genetic polymorphisms include two polymorphisms G>A (rs2275913) and A>T(rs4073) ,and the present results are consistent with results of Kadhim (2023)

The final finding shows that the IL-17A and IL-8 polymorphisms analysis has clear impact on any human diseases. In this study, the polymorphism of IL-17A and IL-8 shows high effect on occurrence of toxoplasmosis and pregnant and aborted women, and the results illustrated in Figures (4-7), (4-8) and Tables (4-7), (4-8) show the role and polymorphism and distribution of IL-17A and IL-8 (rs2275913) G>A and (rs4073) A>T genotypes. The frequency of IL-17A(rs2275913) G>A and A alleles, together with IL-8(rs4073) A>T andT alleles are similar between patients and controls (**Zammiti *et al*., 2006**). In contrast, the frequency of the IL-17A rs2275913 (mutant) G allele (OR of GA=1(0.019-52.03); AA=1.7(0.40-7.3), is higher among patients 18(45%) see Table (4-7).

The other light point in this study, it finds that allele risk of allele A in both heterozygous and homozygous SNP rs2275913 more than risk of allele in SNP rs4073 only with homozygous allele AA=1.7(0.40-7.3) with pregnant and aborted women and *T. gondii* and same with pregnant and aborted women 1.55(0.42-5.7) and 1.7(0.40-7.3) for AT and AA respectively. See Tables (4-7), (4-8).This means SNP: r2275913s G>A more risk than rs4073 A>T on patients under interest.

Conclusions

And

Recommendations

Conclusions and Recommendations

Conclusions

1- Pregnant and aborted women patients have high exposure to toxoplasmosis because of depression of immune system, and age group (18-40) years have high incidence of toxoplasmosis.

2- Patients bear toxoplasmosis shows different percentage, and have the same chance of infection with toxoplasmosis.

3- There are some criteria such as (cat existence in home of infected persons or genetic characters and blood transfusion) have not effected on rates of infection with patients with toxoplasmosis.

4- The IgG predominant in all samples in the present study indicates infection of all patients**.**

5- The IL-17A and IL-8 consider anti-inflammatory or regulatory chemokine.

6- The IL-17A and IL-8 polymorphisms analysis had clear impact on any human diseases, and show high effect on occurrence of toxoplasmosis, polymorphism and distribution of IL-17A and IL-8 rs4073 A>T rs2275913 G>A.

7- The frequency of IL-17A rs2275913 and alleles A, together with IL-8 rs4073 and alleles A, were not similar between patients and controls. Risk allele A more frequent in patients than in control group.

8- The allele frequency of the IL-17A rs2275913 mutant allele A (OR of GA=1(0.019-52.03); AA=1.7(0.40-7.3), were higher among patients 32(80%).

9- The allele frequency of the IL-8 rs4073 mutant allele T (OR of AT=1.55(0.42-5.7)TT=0.53(0.11-2.5),were higher among patients18(45%) .

Recommendations

- Designing specific primers for detecting present of *Toxoplasma gondii* in suspected patients under toxoplasmosis .
- Expanding studies on relationship the parasites with pregnancy and abortion due to spread toxoplasmosis in Iraq.
- Expanding studies that aim to immunogenic explanation of many interleukins like IL-6, IL-12.

References

References

Abdulla, H. E.; Al-bashier, N. M.; Al-kawaz, U.; Al-Shuwaikh, A. M. A. and Abood, A. S. (2015). Cross-sectional study of infertile males with toxoplasmosis in Baghdad Province. Inter. J., Sci. and Eng. Res., 6(1): 254-259.

Al-Dahmoshi, H.O., Al-Mammori, R.T., ShareefHasanain, K., Al-Khafagee Noor, S., 2013. Study of IL-8 and IL-17 levels among certain group of repeated spontaneous abortion women with or without toxoplasmosis, Iraq. Int. Res. J. Biol. Sci. 2 (8), 37–41.

Al-Dalawi, N. K. E. (2007). Hormonal disturbances in suddenly and previously aborted women affected with Toxoplasmosis in Baghdad province. MSc Tech Found Coll Heal Med Technol Tech Found Iraq, 125.

Al-Ddory, A. Z. R. A. (2011). Seroepidemiological study of Toxoplasmosis among pregnant women in Salah–Adden government. *Tikrit Medical Journal*, *17*(1): 64-73.

Al-Dulaimy, S. H. F. (2015). The effect of toxoplasmosis on serum protein components in pregnant and aborted women (Doctoral dissertation, M. Sc. Thesis. College of Science, University of Baghdad,).

Al-Gharrgholi, N. A. S. (2014). Seroepidemiological and Molecular Study for *Toxoplasma gondii* Among Diabetic Mellitus Patientes in Babylon Province M. Sc (Doctoral dissertation, Thesis. College of Science University of Babylon): 128 pp.

Al-Jubori, A. R. (2005). Parasitological and immunological study of *Toxoplasma gondii* in Kirkuk province (Doctoral dissertation, M. Sc. Thesis, College of Medicine, Baghdad University).

References

AL-Jubory, R. J., & IMRAN, Z. K. Molecular Detection of Abundance of Uncultured Lavage Microbiota and Evaluated the SNPs in Partial Sequence of CARD9 Gene.

Al-Jumaili, Z. F. M. (2015). Seroepidemiological and molecular study for toxoplasmosis among blood donors and applicants for marriage and its relationships with testosterone and luteinizing hormones in Babylon Province (Doctoral dissertation, M. Sc. Thesis. College of Science for women, University of Babylon, 99 pp).

Al-Kalaby, R. F. (2008). Sero-epidemiological study of toxoplasmosis among different groups of population in Najaf city (Doctoral dissertation, M. Sc. Thesis. College of medicine. Kufa University).

AL-Maamuri, S. D., AL-Shanawi, F. A., & Melconian, A. K. (2014). Seroprevalence of *Toxoplasma gondii* in Schizophrenic Patients in Iraq using ELISA test. Iraqi Journal of Science, *55*(3B), 1243-1248.

Al-Mousawi, G. N. and Shani, W. S. (2011). Seroepidemiological screening for toxoplasmosis within unmarried women In Basrah Province. *University*

Al-Mayahi, J. R. C. (2011). Epidemiological study on *Toxoplasma gondii* in aborted women in Kut city (Doctoral dissertation, M. Sc. Thesis. College of Science. University of Baghdad): 125 pp of Thi-Qar Journal of Science, 2(4): 34-40.

Al-Mosawi, A. M. A. (2012). Immunoepidemiological study of toxoplasmosis among women in Babylon Province *(Doctoral dissertation* (Doctoral dissertation, Ph. D. Thesis. College of Science. University of Babylon): 120 pp.

References

Al-Obeidi, K. Th. Y. (2004). Toxoplasmosis (cats) in pregnant women and its relationship to some serological variables in women Babylon province M. Sc. Thesis College of Science, University of Mosul: 127 pp.

Al-Rawazq, H. S. A. M. (2017). Seroprevalence of immunoglobulin G (IgG) and immunoglobulin M (IgM) and risk factors of toxoplasmosis for a sample of pregnant women in Baghdad. Al-Kindy College Medical Journal, 13(2): 40-45.

Al-Rawi, K. H. (2009). Detection of B1 gene from blood of pregnant and abortive women infected with *Toxoplasma gondii*. (Doctoral dissertation, Ph. D. Thesis). College of Science. University of Baghdad, 125pp.

Al-Saadii, S. H. (2013). The effect of toxoplasmosis on the level of some male sex hormones in samples from National blood transfusion center/Baghdad (Doctoral dissertation, M. Sc. thesis, College of Science, University of Baghdad, Iraq).

Al-Shikhly, M. A. (2010). Early detection of toxoplasmosis percentage in pre-marital females by immunological methods (Doctoral dissertation, M.Sc. Thesis. College of Science. Baghdad University), 147 pp.

Ali, M. I., Abd El Wahab, W. M., Hamdy, D. A., & Hassan, A. (2019). *Toxoplasma gondii* in cancer patients receiving chemotherapy: seroprevalence and interferon gamma level. Journal of Parasitic Diseases, 43, 464-471.

Ali, S., Majid, S., Ali, M., Banday, M. Z., Taing, S., Wani, S., ... & Rehman, M. U. (2022). Immunogenetic Role of IL17A Polymorphism in the Pathogenesis of Recurrent Miscarriage. Journal of Clinical Medicine, 11(24), 7448.

References

Ali, Z., Hossein, M. M., and Khadijeh, D. (2007). Toxoplasma chorioretinitis in primary school children in Tehran, Iran, 2003-2004. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research, 13(4): CR201-205.

Amissah-Reynolds, P. K. (2020). Zoonotic risks from domestic animals in Ghana. International Journal of Pathogen Research, 4(3): 17-31.

Angel, S. O., Vanagas, L., Ruiz, D. M., Cristaldi, C., Saldarriaga Cartagena, A. M., and Sullivan Jr, W. J. (2020). Emerging therapeutic targets against *Toxoplasma gondii*: update on DNA repair response inhibitors and genotoxic drugs. Frontiers in Cellular and Infection Microbiology, 10, 289.

Anselmo, L. M. P., Vilar, F. C., Lima, J. E., Yamamoto, A. Y., Bollela, V. R., & Takayanagui, O. M. (2014). Usefulness and limitations of polymerase chain reaction in the etiologic diagnosis of neurotoxoplasmosis in immunocompromised patients. Journal of the Neurological Sciences, 346(1-2), 231-234.

Attias, M.; Teixeira, D.E.; Benchimol, M.; Vommaro, R.C.; Crepaldi, P.H.; De Souza, W. (2020). The Life-Cycle of Toxoplasma Gondii Reviewed Using Animations. Parasites Vectors. 13, 588.

Abaie, J.; Amiri, S.; Mostafavi, E.; Hassan, N.; Lotfi, P.; Rastaghi, A. R. E. and Golkara, M. (2013). Seroprevalence and risk factors for *Toxoplasma gondii* infection among pregnant women in northeast Iran. Clin. Vaccin. Immunol., 20(11): 1771 - 1773.

Bahia-Oliveria, L. M. G., Jones, J. L., Azevedo-Silva, J., Alves, C. C., Oréfice, F., and Addiss, D. G. (2003). Highly endemic, waterborne toxoplasmosis

in north Rio de Janeiro state, Brazil. Emerging infectious diseases, 9(1): 55.

Balkwill, F. (2004). Cancer and the chemokine network. Nature reviews cancer, 4(7), 540-550.

Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D. S., ... & Zychlinsky, A. (2004). Neutrophil extracellular traps kill bacteria. science, 303(5663), 1532-1535.

Caballero-Campo, P., Domínguez, F., Coloma, J., Meseguer, M., Remohí, J., Pellicer, A., & Simón, C. (2002). Hormonal and embryonic regulation of chemokines IL-8, MCP-1 and RANTES in the human endometrium during the window of implantation. Molecular human reproduction, 8(4), 375-384.

Cerutti, A., Blanchard, N., & Besteiro, S. (2020). The bradyzoite: a key developmental stage for the persistence and pathogenesis of toxoplasmosis. Pathogens, 9(3), 234.

Costa-Silva, T. A., Borges, M. M., Galhardo, C. S., and Pereira-Chioccola, V. L. (2012). Immunization with excreted/secreted proteins in AS/n mice activating cellular and humoral response against *Toxoplasma gondii* infection. *Acta tropica*, *124*(3): 203-209.

Crawford, D. C., & Nickerson, D. A. (2005). Definition and clinical importance of haplotypes. Annu. Rev. Med., 56, 303-320.

Daher, S., Shulzhenko, N., Morgun, A., Mattar, R., Rampim, G. F., Camano, L., and DeLima, M. G. (2003). Associations between cytokine gene polymorphisms and recurrent pregnancy loss. Journal of reproductive immunology, 58(1): 69-77.

References

Dubey. J. P. (2007). The history and life cycle of *Toxoplasma gondii* In : loynson, D. H. M. and Wreghitt, T. G. (Eds.). Toxoplasmosis acomprehensive clinical guide, 3rd edn., Cambridge University press, London: 1-17 pp.

Dubey, J.P. (2014). The history and life cycle of *Toxoplasma gondii*. In *Toxoplasma Gondii*; Elsevier: Amsterdam, The Netherlands pp. 1–17.

Deshpande, P. S., Kotresha, D., Noordin, R., Yunus, M. H., Saadatnia, G., Golkar, M., ... & Ghaffarifar, F. (2013). IgG avidity Western blot using Toxoplasma gondii rGRA-7 cloned from nucleotides 39-711 for serodiagnosis of acute toxoplasmosis. Revista do Instituto de Medicina Tropical de São Paulo, 55, 79-83.

Dubey, J. P. (2014). In *Toxoplasma gondii*: the model apicomplexan - perspectives and methods (eds L. M. Weiss and K. Kim) 1–17 (Academic Press London, Waltham and San Diego).

Dubey, J.P.; Murata, F.H.A.; Cerqueira-Cézar, C.K.; Kwok, O.C.H.(2020). Public health and economic importance of Toxoplasma Gondii Infections in Goats: The Last Decade. Res. Vet. Sci. 132, 292–307.

Ebrahimzadeh, A., Mohammadi, S., SalimiKhorashad, A., and Jamshidi, A. (2013). Seroprevalence of Toxoplasmosis among pregnant women referring to the reference laboratory of zahedan, Iran. Zahedan Journal of Research in Medical Sciences, 15(12): e4332.

Elnahas, A., Gerais, A. S., Eibashir, M. I., Eldien, E. S. and Adam, I. (2003). Toxoplasmosis in pregnant Sudanese women. Saudi medical journal, 24(8): 868-870.

References

Ferguson, D.J. (2009). "Toxoplasma gondii: 1908–2008, homage to Nicolle, Manceaux and Splendore". Memórias do Instituto Oswaldo Cruz. 104 (2): 133–48.

Ferguson, D.J.P.; Dubremetz, J.-F. (2014). The Ultrastructure of *Toxoplasma gondii*. In *Toxoplasma Gondii*; Weiss, L.M., Kim, K., Eds.; Elsevier: Amsterdam, The Netherlands, pp. 19–59.

Flegr, J., Prandota, J., Sovičková, M., and Israili, Z. H. (2014). Toxoplasmosis–a global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. *PloS one*, *9*(3): e90203.

Gaetano, P., Angelina, C. and Michele, R. (2010). Acute pericar- ditis and myocarditis *Toxoplasma gondii* in an immune man, a case report. J. Infect. Med, 18(1): 48-52.

Galal L, Hamidović A, Dardé ML, *et al*. (2019). Diversity of *Toxoplasma gondii* strains at the global level and its determinants. Food Waterborne Parasitol. 15:e00052.

Galván-Ramírez Mde L, Gutiérrez-Maldonado AF, Verduzco-GrijalvaF, Jiménez JM(2014) The role of hormones on *Toxoplasma gondii* infection: a systematic review.Front Microbiol. 5: 503.

Galvan-Rameriz M, Sanchez-Orozcol L, Ramirez Cynthia (2017). The laboratory diagnosis in *toxoplasma* infection. SN: 978-953-51-3269-1.

Gazzinelli, R. T., Mendonca-Neto, R., Lilue, J., Howard, J. and Sher, A. (2014). Innate resistance against *Toxoplasma gondii*: an evolutionary tale of mice, cats, and men. Cell host and microbe, 15(2): 132-138.

Gerhardt, K. E., Huang, X. D., Glick, B. R., & Greenberg, B. M. (2009). Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. Plant science, 176(1), 20-30.

References

Ghasemian, M., Maraghi, S. H., Saki, J., & Pedram, M. (2007). Determination of antibodies (IgG, IgM) against *Toxoplasma gondii* in patients with cancer.

Ghosh, S. (2013) . Paniker's textbook of Medical parasitology, Seven edition. Jaypee Brothers medical publishers (P) LTD. ISBN 978-93-5090-534-0. 276 pp.269.

Gissot, M. (2022). *Toxoplasma gondii*: Asexual Cycle in the Intermediate Host. In Lifecycles of Pathogenic Protists in Humans (pp. 391-417). Springer, Cham.

Gómez-De León C.T., Mondragón-Flores R. (2017). "2: Morphology".In Galán Ramírez M.dl.L.Mondragón-Flores R., ed. Human Toxoplasmosis (PDF). Research Gate. p. 11.

Gonzalez-Aparicio, M., & Alfaro, C. (2019). RETRACTED ARTICLE: Significance of the IL-8 pathway for immunotherapy. Human vaccines & immunotherapeutics, 16(10), 2312-2317.

Gotteland, C., Gilot-Fromont, E., Aubert, D., Poulle, M. L., Dupuis, E., Dardé, M. L., and Villena, I. (2014). Spatial distribution of *Toxoplasma gondii* oocysts in soil in a rural area: Influence of cats and land use. Veterinary Parasitology, 205(3-4): 629-637.

Gubbels, M. J., Keroack, C. D., Dangoudoubiyam, S., Worliczek, H. L., Paul, A. S., Bauwens, C., ... &Duraisingh, M. T. (2020). Fussing about fission: defining variety among mainstream and exotic apicomplexan cell division modes. Frontiers in cellular and infection microbiology, 10.

Ha, H., Debnath, B., & Neamati, N. (2017). Role of the CXCL8-CXCR1/2 axis in cancer and inflammatory diseases. Theranostics, 7(6), 1543.

References

Halonen, S.K. and Weiss, L.M. (2013). "Toxoplasmosis," in Neuroparasitology and Tropical Neurology, vol. 114 of Handbook of Clinical Neurology, pp. 125–145, Elsevier.

Hamza, J. K. (2006). Seroepidemiological study of *Toxoplasma* antibodies among women in reproductive age in Hilla city (Doctoral dissertation, M. Sc. Thesis. College ofUnivercity of Baghdad pp102).

Hedrick, P. W. (2013). Genetics of populations. Jones & Bartlett Publishers.

Hegab, S. M. and Al-Mutawa, S. A. (2003). Immunopathogenesis of toxoplasmosis. Clinical and experimental medicine, *3*(2): 84-105.

Hill, S. E. and Dubey, J. P. (2014). Toxoplasmosis: Reston, Va., U.S. Geological Survey Circular, 1389, 84.

Hunter, C.A.; Sibley, L.D. (2012). Modulation of innate immunity by *Toxoplasma gondii* virulence effectors. Nat. Rev. Microbiol. 10, 766–

778.

Hussein, H. E., Bastos, R. G., Schneider, D. A., Johnson, W. C., Adham, F. K., Davis, W. C., and Suarez, C. E. (2017). Te Babesia bovis hap2 gene is not required for blood stage replication, but expressed upon in vitro sexual stage induction. PLoS Neglected Tropical Diseases, 11(10): e0005965

Hymowitz, S. G., Filvaroff, E. H., Yin, J., Lee, J., Cai, L., Risser, P., ... & Starovasnik, M. A. (2001). IL-17s adopt a cystine knot fold: structure and activity of a novel cytokine, IL-17F, and implications for receptor binding. The EMBO journal, 20(19), 5332-5341.

References

Iqbal, J., and Khalid, N. (2007). Detection of acute *Toxoplasma gondii* infection in early pregnancy by IgG avidity and PCR analysis. *Journal of Medical* Microbiology, 56(11): 1495-1499.

Jassam, F. S. (2010). Relationship between toxoplasmosis and testosterone hormone among schizophrenic patients in Baghdad (Doctoral dissertation, Dissertation, College Council of Health and Medical Technology. Baghdad, Iraq).

Jones, J. L., Kruszon-Moran, D., Wilson, M., McQuillan, G., Navin, T., and McAuley, J. B. (2001). *Toxoplasma gondii* infection in the United States: seroprevalence and risk factors. American journal of epidemiology, 154(4): 357-365.

Jones, J. L., Muccioli, C., Belfort Jr, R., Holland, G. N., Roberts, J. M., and Silveira, C. (2006). Recently acquired *Toxoplasma gondii* infection, Brazil. Emerging infectious diseases, 12(4): 582-782.

Kadir, M. A., Ghalib, A. K., Othman, N. F. and Ahmed, I. S. (2011). Seroprevalence of *Toxoplasma gondii* among pregnant women in Kirkuk / Iraq. Journal of Kirkuk University–Scientific Studies, 6(2): 1-5.

Kadhim, R. (2007). The role of some immunological changes and some viral infection in single and recurrent spontaneous abortion. Ph. D. Thesis, College of science, University of Baghdad. 120pp.

Kadhum, L. S., Rabee, A. K. A., & Kadhim, A. J. (2023). *Toxoplasma gondii* infection correlated to Interleukin 12 in wasit Women. Journal of Survey in Fisheries Sciences, 10(3S), 592-597.

Kamali-Sarvestani, E., Zolghadri, J., Gharesi-Fard, B., and Sarvari, J. (2005). Cytokine gene polymorphisms and susceptibility to recurrent pregnancy loss in Iranian women. Journal of reproductive immunology, 65(2): 171-178.

References

Kang, H. C., Kim, I. J., Park, J. H., Shin, Y., Ku, J. L., Jung, M. S., ... & Park, J. G. (2004). Identification of genes with differential expression in acquired drug-resistant gastric cancer cells using high-density oligonucleotide microarrays. Clinical Cancer Research, 10(1), 272-284.

Kareem, S. S. (2008). Prevalence, serodiagnosis and some immunological aspects of toxoplasmosis among women in Baghdad province (Doctoral dissertation, M. Sc. Thesis. College of health and Medical Technology. Technical Foundation): 104 pp.

Khalil, H. I. (2008). Some aspects in Seroprevalence, diagnosis and influence of sex hormones on immunity during human toxoplasmosis (Doctoral dissertation, Ph. D. Thesis. College of Medicine, University of Al-Mustansiriyah): 173 pp.

Knoll L. J., Tomita T., Weiss L. M. (2013). "Chapter 15 - BradyzoiteDevelopment," in *Toxoplasma Gondii:* The Model Apicomplexan -Perspectives and Methods: Second Edition. Eds. Weiss L. M., Kim K. (Academic Press), 521–549.

Kolls, J.K., and Linde´ n, A. (2004). Interleukin-17 family members and inflammation. Immunity 21, 467–476.

Kolören, Z. and Dubey, J.P. (2020) A review of toxoplasmosis in humans and animals in Turkey. *Parasitology*, 147(1): 12-28.

Larosa, D. F., Stumhofer, J. S., Gelman, A. E., Rahman, A. H., Taylor, D. K., Hunter, C. A., and Turka, L. A. (2008). T cell expression of MyD88 is required for resistance to *Toxoplasma gondii*. Proceedings of the National Academy of Sciences, 105(10): 3855-3860.

References

Li, L., Li, J., Gao, M., Fan, H., Wang, Y., Xu, X., ... & Yang, H. (2021). Interleukin-8 as a biomarker for disease prognosis of coronavirus disease-2019 patients. Frontiers in immunology, 11, 602395.

Lieberman, L. A., & Hunter, C. A. (2002). The role of cytokines and their signaling pathways in the regulation of immunity to *Toxoplasma gondii*. International reviews of immunology, 21(4-5), 373-403.

Liu Q, Wang Z-D, Huang S-Y, Zhu X-Q. (2015). Diagnosis of toxoplasmosis and typing of *Toxoplasma gondii*. Parasites & Vectors. 8:(292):PMID: 26017718.

Mahadevan, A., Ramalingaiah, A. H., Parthasarathy, S., Nath, A., Ranga, U., & Krishna, S. S. (2013). Neuropathological correlate of the "concentric target sign" in MRI of HIV-associated cerebral toxoplasmosis. Journal of Magnetic Resonance Imaging, 38(2), 488-495.

Mahami-Oskouei, M., Moradi, M., Fallah, E., Hamidi, F., and Akbari, N. A. R. (2017). Molecular detection and genotyping of *Toxoplasma gondii* in chicken, beef, and lamb meat consumed in Northwestern Iran. Iranian journal of parasitology, 12(1): 38.

Mahmood, S. H., AL-Qadhi, B. N., & Zghair, K. H. (2013). Prevalence of toxoplasmosis of males blood donors in Baghdad. Iraqi J Sci, 54(4), 832-41.

Mahmood, T. A. (2014). Serological and molecular comparative diagnosis of Toxoplasmosis and certain types of viral infection. Ph. D. Thesis, college of medicine, Kufa University: 123 pp.

Mohammed, G. J. A. (2008). Study the role Toxoplasmosis, cytomegalovirus and anti-phospholipids antibodies in cases abortionamong women in Hilla city". M. Sc. thesis. College Medicine. Babylion Univeristy :123 pp.

References

Mohammed, K. G., Baqur, A., Al, A. P. D. S. A., & Al-Sagheer, H. A. (2012). The role T-helper-17 in toxoplasmosis among women with abortion. KUFA MEDICAL JOURNAL, 15(1).

Maleki, B., Ahmadi, N., Olfatifar, M., Gorgipour, M., Taghipour, A., Abdoli, A., ... & Mirzapour, A. (2021). *Toxoplasma* oocysts in the soil of public places worldwide: a systematic review and meta-analysis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 115(5), 471-481.

Marcuzzi, E., Angioni, R., Molon, B., & Calì, B. (2018). Chemokines and chemokine receptors: orchestrating tumor metastasization. International journal of molecular sciences, 20(1), 96.

Mekori, Y. A., & Metcalfe, D. D. (2000). Mast cells in innate immunity. Immunological reviews, 173(1), 131-140.

Miller, C. M., Boulter, N. R., Ikin, R. J., and Smith, N. C. (2009). The immunobiology of the innate response to *Toxoplasma gondii*. International journal for parasitology, 39(1): 23-39.

Manouchehri Naeini, K., Heidari Soureshjani, E., Jafari, M., Parchami, S., Karimi, G., and Abdizadeh, R. (2019). Prevalence of *Toxoplasma gondii* Infection in Healthy Volunteer Blood Donors Using Serological and Molecular Methods from Chaharmahal and Bakhtiari Province, Southwest Iran. Jundishapur Journal of Microbiology, 12(5): e91042

McAuley JB. (2014). Congenital toxoplasmosis. J Pediatric Infect DisSoc. 3(Suppl 1):S30–35.

Montazeri, M., Mikaeili Galeh, T., Moosazadeh, M., Sarvi, S., Dodangeh, S., Javidnia, J. and Daryani, A. (2020). The global serological prevalence of *Toxoplasma gondii* in felids during the last five decades (1967–2017): a systematic review and meta-analysis. *Parasites and vectors*, *13*(1): 1-10.

References

Moore, K. W., de Waal Malefyt, R., Coffman, R. L., and O'Garra, A. (2001). Interleukin-10 and the interleukin-10 receptor. Annual review of immunology, 19(1): 683-765.

Moore, B. B., & Kunkel, S. L. (2019). Attracting attention: discovery of IL-8/CXCL8 and the birth of the chemokine field. The Journal of Immunology, 202(1), 3-4.

Ngô, H. M., Zhou, Y., Lorenzi, H., Wang, K., Kim, T. K., Zhou, Y., ... & McLeod, R. (2017). *Toxoplasma* modulates signature pathways of human epilepsy, neurodegeneration & cancer. Scientific Reports, 7(1), 11496.

Nimri, L., Pelloux, H. and El-Khatip, L. (2004). Detection of *Toxoplasma gondii* DNA and specific antibodies in high – risk pregnant women. *The* American journal of tropical medicine and hygiene, 71(6): 831-835.

Omidian, M., Ganjkarimi, A. H., Asgari, Q., and Hatam, G. (2021). Molecular and serological study on congenital toxoplasmosis in newborn of Shiraz, Southern Iran. Environmental Science and Pollution Research, 28(13): 16122-16128.

Panah, A. S., Assadi, M., Soufiani, K., Barzegar, G., Gharachorlou, A., & Emami Zeyd, A. (2013). Seroprevalence of *Toxoplasma gondii* infection among pregnant women in Amol, Northern Iran. Life Science Journal, 10(2s).

Park Y-H, Nam H-W. (2013). Clinical features and treatment of ocular toxoplasmosis. Korean J Parasitol. 51 (4):393–399.

Payne, A. S., & Cornelius, L. A. (2002). The role of chemokines in melanoma tumor growth and metastasis. Journal of Investigative Dermatology, 118(6), 915-922.

References

Petersen, E. (2007). Toxoplasmosis. Seminars in Fetal and Neonatal Medicine, 12(3): 214-223.

Pinto-Ferreira, F., Caldart, E. T., Pasquali, A. K. S., Mitsuka-Breganó, R., Freire, R. L., and Navarro, I. T. (2019). Patterns of transmission and sources of infection in outbreaks of human toxoplasmosis. Emerging infectious diseases, 25(12): 2177-2182.

Roberts, L. S., & Janovy Jr, J. (2000). Gerald D. Schmidt e Larry S. Roberts' foundations of parasitology. In Gerald D. Schmidt e Larry S. Roberts' foundations of parasitology (pp. xviii-670).

Robert-Gangneux F and Darde ML (2012). "Epidemiology of anddiagnostic strategies for toxoplasmosis," Clinical Microbiology Reviews,vol. 25, no. 2, pp. 264–296.

Sadiqui, S., Shah, S. R. H., Almugadam, B. S., Shakeela, Q., and Ahmad, S. (2018). Distribution of *Toxoplasma gondii* IgM and IgG antibody seropositivity among age groups and gestational periods in pregnant women. F1000Research, 7: 1823

Saleh, M. A. (2011). Determination of antibodies (IgG, IgM) against *Toxoplasma gondii* in some Iraqi individuals by using ELISA technique. Baghdad Science Journal, 8(4), 940.

Salman, Y. J. (2014). Watching of *Toxoplasma gondii* antibodies among peoples in Kirkuk Province from 1993 to 2012 by using different serological tests. Int. J. Curr. Microbiol. App. Sci. 3(9): 923-932.

Sasai, M., Yamamoto, M. (2019). Innate, adaptive, and cell-autonomous immunity against *Toxoplasma gondii* infection. Exp Mol Med 51, 1–10.

References

Secrieru, A., Costa, I. C., O'Neill, P. M., and Cristiano, M. L. (2020). Antimalarial Agents as Therapeutic Tools Against Toxoplasmosis—A Short Bridge between Two Distant Illnesses. Molecules, 25(7): 1574.

Shapiro, K.; Bahia-Oliveira, L.; Dixon, B.; Dumètre, A.; deWit, L.A.;VanWormer, E.; Villena, I. (2019). Environmental Transmission of *Toxoplasma gondii:* Oocysts in Water, Soil and Food. Food Waterborne Parasitol. 15, e00049.

Shin, D. W., Cha, D. Y., Hua, Q. J., Cha, G. H., and Lee, Y. H. (2009). Seroprevalence of *Toxoplasma gondii* infection and characteristics of seropositive patients in general hospitals in Daejeon, Korea. The Korean journal of parasitology, 47(2): 125-130.

Shiojiri, D., Kinai, E., Teruya, K., Kikuchi, Y., and Oka, S. (2019). Combination of clindamycin and azithromycin as alternative treatment for *Toxoplasma gondii* encephalitis. Emerging infectious diseases, 25(4): 841.

Sinai A. P., Watts E. A., Dhara A., Murphy R. D., Gentry M. S.,Patwardhan A. (2017). Activity for a " Dormant " Parasite. Curr. Clin.Microbiol. Rep. 3, 175–185.

Singh, S. and Pandit, A. J. (2004). Incidence and prevalence of toxoplasmosis in Indian pregnant women: a prospective study. American Journal of Reproductive Immunology, 52(4): 276-283.

Skov, L., Beurskens, F. J., Zachariae, C. O., Reitamo, S., Teeling, J., Satijn, D., ... & van de Winkel, J. G. (2008). IL-8 as antibody therapeutic target in inflammatory diseases: reduction of clinical activity in palmoplantar pustulosis. The Journal of Immunology, 181(1), 669-679.

References

Stajner T, Vasiljević Z, Vujić D, Marković M, Ristić G, Mićić D,Pasić S, Ivović V, Ajzenberg D, Djurković-Djaković O. (2013).Atypical strain of *Toxoplasma gondii* causing fatal reactiva tion after hematopoietic stem cell transplantion in a patient with an underlying immunological de ciency. J. Clin. Microbiol. 51:(2686–2690):PMID: 23761151.

Stelzer, S.; Basso, W.; Benavides Silván, J.; Ortega-Mora, L.M.;Maksimov, P.; Gethmann, J.; Conraths, F.J.; Schares, G. (2019) *Toxoplasma gondii* infection and toxoplasmosis in farm animals: Risk factors and economic impact. Food Water b. Parasitol. 15, e00037.

Syrian Sanchez, Sébastien Besteiro. (2021). The pathogenicity and virulence of Toxoplasma gondii. Virulence, Taylor & Francis. 12 (1), pp.3095 - 3114.

Tawfiq , S. K., Rezaig , Z.S. and Al- Jebouri , M. M. (2019). Determination the Time of Toxoplasmosis among Pregnant Women by using IgG Avidity to Various *Toxoplasma gondii* Antigens. Kirkuk University Journal-Scientific Studies, 14(1): 1-12.

Tenter, A. M ; Heckeroth, A. R. and Weiss, L. M. (2000). *Toxoplasma gondii*: from animals to humans. International journal for parasitology, 30(12-13): 1217-1258.

Tomita T, Bzik DJ, Ma YF, Fox BA, Markillie LM,Taylor RC, Kim K,and Weiss LM (2013). "The *Toxoplasma gondii* Cyst Wall Protein CST1 Is Critical for Cyst Wall Integrity and Promotes Bradyzoite Persistence", PLoS Pathogens, vol. 9, pp. e1003823.

Tomita T, Guevara RB, Shah LM, *et al*. (2021). Secreted effectorsmodulating immune responses to *Toxoplasma gondii*. Life. 11(9):988.

References

Tonkal, A. M. (2008). PCR versus ELISA in diagnosis of human toxoplasmosis in Jeddah, Saudi Arabia. J Egypt Soc Parasitol, 38(3): 707-714.

Tyebji S, Seizova S, Hannan AJ, *et al.* (2019). Toxoplasmosis: a pathway to neuropsychiatric disorders. Neurosci Biobehav Rev. 96:72–92.

Walker, R. A., Sharman, P. A., Miller, C. M., Lippuner, C., Okoniewski, M., Eichenberger, R. M., and Smith, N. C. (2015). RNA Seq analysis of the Eimeria tenella gametocyte transcriptome reveals clues about the molecular basis for sexual reproduction and oocyst biogenesis. BMC genomics, 16(1): 1-20.

Wang, Z. D., Wang, S. C., Liu, H. H., Ma, H. Y., Li, Z. Y., Wei, F., ... & Liu, Q. (2017). Prevalence and burden of *Toxoplasma gondii* infection in HIV-infected people: a systematic review and meta-analysis. *The Lancet HIV*, *4*(4), e177-e188.

Weaver, C.T., Hatton, R.D., Mangan, P.R., and Harrington, L.E. (2007). IL-17family cytokines and the expanding diversity of effector T cell lineages. Annu. Rev. Immunol. 25, 821–852.

Weiss, L.M.and Dubey, J.P. (2009). "Toxoplasmosis: A history of clinical observations". International Journal for Parasitology. 39 (8): 895–901.

White, Michael W., and Elena S. Suvorova. (2018). "Apicomplexa cellcycles: something old, borrowed, lost, and new." *Trends in parasitology* 34.9 : 759-771.

Wright, J.F., Guo, Y., Quazi, A., Luxenberg, D.P., Bennett, F.,and Ross,J.F.(2007). Identification of an interleukin 17F/17A heterodimer in activated human CD4+ T cells. J Biol Chem,282:13447–13455.

References

Yacoub, A. A. H., Bakr, S., Hameed, A. M., Al Thamery, A. A. A., and Fartoci, M. J. (2006). Seroepidemiology of selected zoonotic infections in Basra region of Iraq. EMHJ-Eastern Mediterranean Health Journal, 12 (1-2): 112-118.

Yamagish J, Suzuki Y. (2015). The genome-wide identi cation ofpromoter regions in *Toxoplasma gondii*. Methods Mol. Biol. 1201:(193–205):PMID: 25388115.

Ybanez,R.H.D.,Ybanez,A.P.and Nishikawa,Y.(2020).Review on the current trends of toxoplasmosis serodiagnosis in humans. Front.cell infect.Microbiol.,10(204): 1-8.

Yobi, D.; Piarroux, R.; L'Ollivier, C.; Franck, J.; Situakibanza, H.; Muhindo, H.; Mitashi, P.; Luz, R. A. I. D.; Sprundel, M. V.; Boelaert, M.; Geertruyden, J. V. and Lutumba, P. (2014). Toxoplasmosis among pregnant women: High seroprevalence and risk factors in Kinshasa, Democratic Republic of Congo. Asian, Pac. J. Trop Biomed., 4(1): 69-74.

Zainodini, N., Mohammad, Z. B., Abdollahi, S. H., Afrooz, M., Ziaali, N., Ebrahimian, M., & Arababadi, M. K. (2014). Molecular and serological detection of acute and latent toxoplasmosis using real-time PCR and ELISA techniques in blood donors of Rafsanjan City, Iran, 2013. Iranian Journal of Parasitology, 9(3), 336. Al-Dahmoshi, H.O., Al-Mammori, R.T., ShareefHasanain, K., Al-Khafagee Noor, S., 2013. Study of IL-8 and IL-17 levels among certain group of repeated spontaneous abortion women with or without toxoplasmosis, Iraq. Int. Res. J. Biol. Sci. 2 (8), 37–41

Appendices

Appendix

Appendix (1):questionnaire sheet that provided for all patients in the present study.

Sample collection form	
Name	
Age	
Region (urban – rural)	
Sample collection date	
Blood group	
Are cats in the house or not	
Eating meat (raw - undercooked - eating canned meat)	
Abortion No	
Duration of pregnancy	

Appendix (2): The ethical paper given to pregnant and aborted women to take agree or not to take blood samples.

Paper that reveal the agreement of patient before taken the samples as ethics.

Ethics

You are invited (e) to participate in the clinical scientific research that will be conducted at ---------- Please take (j) enough time to read the following information carefully before deciding (j) To participate or not. You can request clarifications or additional information about anything in this form or about this study from your doctor.

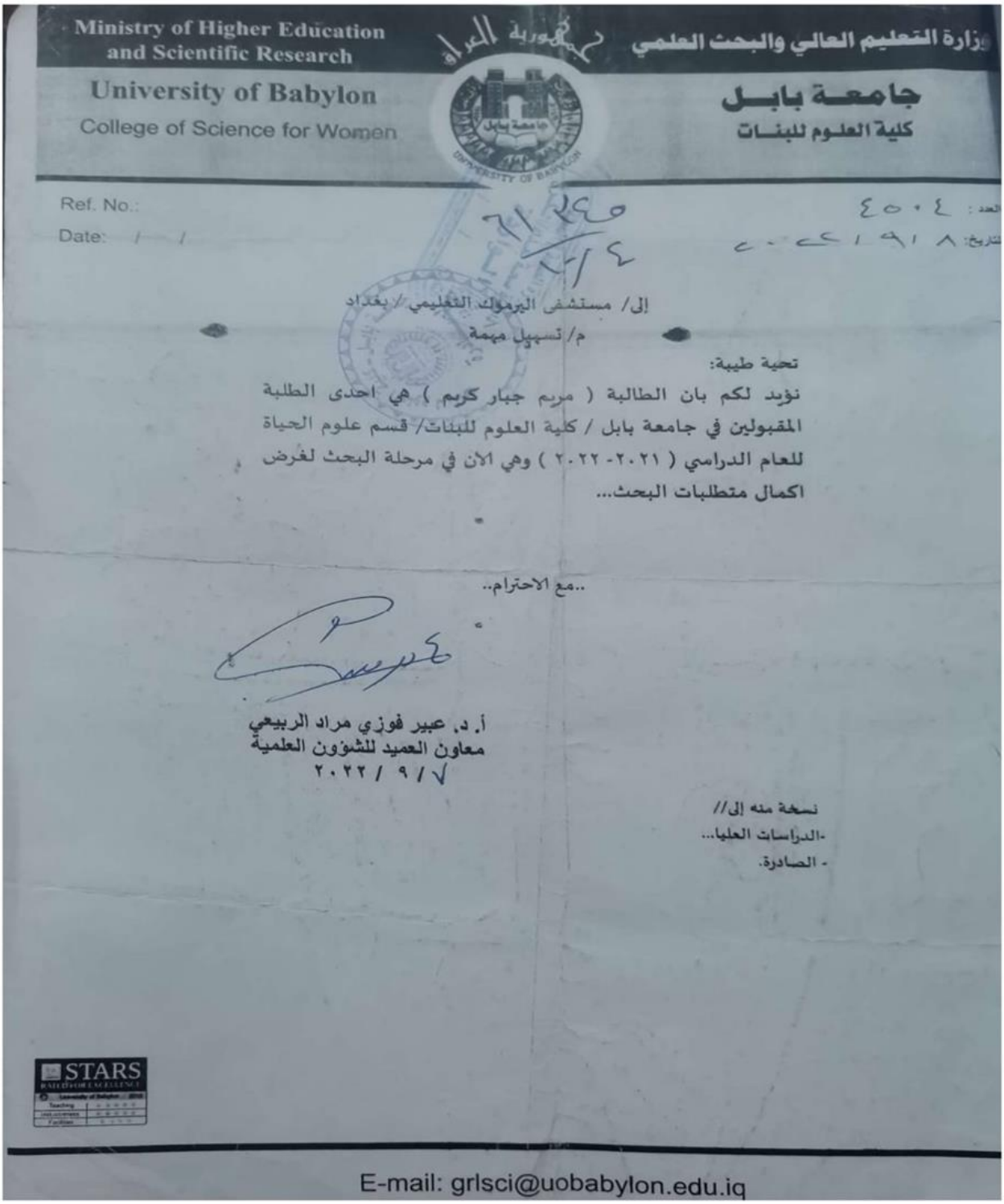
In the event that you agree and accept to participate in this study, your name, address and phone number will remain.

Agree ☐ **Not agree** ☐

I have read this acceptance form and know what it is about. All my questions were answered. I authorize the conduct of this study and agree to participate in it,

Subscriber name: **Subscriber signature:**

Appendix



Ministry of Higher Education and Scientific Research
University of Babylon
College of Science for Women

وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية العلوم للبنات

Ref. No.:
Date: / /

العدد: ٤٠٥٤
التاريخ: ٢٠٢٢/٩/٨

إلى/ مستشفى اليرموك التعليمي / بغداد
م/ تسهيل مهمة

تحية طيبة:

نؤيد لكم بان الطالبة (مريم جبار كريم) هي احدى الطلبة المقبولين في جامعة بابل / كلية العلوم للبنات/ قسم علوم الحياة للعام الدراسي (٢٠٢١-٢٠٢٢) وهي الان في مرحلة البحث لغرض اكمال متطلبات البحث...

..مع الاحترام..

أ. د. عبير فوزي مراد الربيعي
معاون العميد للشؤون العلمية
٢٠٢٢ / ٩ / ٨

نسخة منه إلى//
-الدراسات العليا...
- الصادرة.

STARS

E-mail: grlsci@uobabylon.edu.iq

Appendix (3): A letter facilitating a task to the Al-Yarmok teaching \Baghdad for the purpose of examining samples pregnant and aborted women .

الخلاصـــــة

تهدف الدراسة الحالية إلى تقييم داء المقوسات في النساء الحوامل والمجهضات بناءً على معايير مناعية بواسطة الاضداد IgG و IgM والمعايير الوبائية ومن خلال الكشف عن تعدد أشكال الجين انترلوكين-8 وانترلوكين- 17aوبيان علاقتها بداء المقوسات.

تم إجراء تجارب مختبرية لدراسة 96 عينة تم جمعها لنساء حوامل ومجهضات ، وتم فحص هذه العينات في جهاز VIDAS لتعرف على الاضداد IgG و IgM ، وتم الحصول على 20عينة موجبة لاختبار IgG من النساء الحوامل والمجهضات 14عينه كانت موجبه لضد IgG لنساء حوامل و6عينه موجبه لاختبار لضد IgG لنساء مجهضات , ولا يوجد عينة منها موجبة لضد IgM.

وبحسب تقسيم عينات الدراسة إلى ست فئات عمرية ،من 96 عينه من نساء حوامل ومجهضات تظهر نتائج النسبة المئوية (18.5%)في الفئات العمرية(22-18) سنة للنساء الحوامل والمجهضات المصابات بداء المقوسات، بينما تبلغ النسبة (18.18%) في الفئات العمرية(27-23) سنة. بينما النسبة المئوية(25%)في الفئات العمرية (32-28%) سنة ، النسبة مئوية كانت(18.75%) في الفئات العمرية(37-33) سنة, وكانت اعلى نسبه كانت (26.66%) في الفئات العمرية (42-38) سنة و(0%) في الفئة العمرية(43واكثر) سنة في النساء الحوامل و المجهضات كانت الاجسام المضادة لضد IgG الموجب للمصابات بداء القطط,وكانت النسبة المئوية (0%) لضد IgM .

وبحسب تقسيم عينات الدراسة حسب منطقة الاقامة(ريف , مدينة) تظهر نتائج النسبة المئوية (31.11%) موجبة للضد IgG للنساء الحوامل والمجهضات في الريف، بينما تبلغ النسبة (11.76%) موجبة لضدIgG للنساء الحوامل والمجهضات في المدينة, وكانت النسبة المئوية (0%) لضد IgM .

وبحسب تقسيم عينات الدراسة ل96عينه من نساء حوامل ومجهضات حسب وجود القطط قسمت عينات الدراسة حسب وجود القطط الى اثنان(القطط موجودة والقطط غير موجودة) تضهر النتائج النسبة المئوية(21.42%) كانت الاجسام المضادة IgG موجبة لداء القطط بجود القطط في النساء الحوامل والمجهضات, النسبة المئوية (20%) كانت موجبة للاجسام المضادة IgG بداء المقوسات عندما تكون القطط غير الموجودة في مرضى داء المقوسات كانت (0%)موجبه لضد IgM لكل العينات.

وبحسب تقسيم عينات الدراسة حسب عدد مرات الاجهاض إلى ثلاث فئات (مره واحدة,مرتين ،ثلاث مرات واكثر) من النساء المجهضات تظهر نتائج النسبة المئوية(33.33%) موجبة لضد IgG في النساء المجهضات مرة احدة، بينما تبلغ النسبة (50%) موجبة لضد IgG في النساء المجهضات مرتين . بينما النسبة المئوية (30%) لضدIgG في النساء المجهضات ثلاث مرات واكثر، وكانت النسبة المئوية (0%) لضد IgM .

وبحسب تقسيم عينات الدراسة حسب فترة الحمل إلى ثلاث فئات (1-3),(4-6),(7-9) من النساء الحوامل تظهر نتائج النسبة المئوية (9.09%) موجبة لضد IgG في النساء الحوامل في الفترة(1–3)، بينما تبلغ النسبة (20%) موجبة لضد IgG في النساء االحوامل في الفترة(4–6) . بينما النسبة المئوية (19.04%) لضدIgG في النساء الحوامل في الفترة(7–9)، وكانت النسبة المئوية (0%) لضد IgM .

هذا يعني ان لضد نوع IgG هو السائد في الدراسة الحالية ,ولضد نوع IgM كانت مفقودة دلالة ان الاصابات كانت مزمنه.

نتائج التنميط الجيني للأليلات البرية والمتحورة لتعدد أشكال النوكليوتيدات المفردة لجين إنترلوكين–17A وانترلوكين–8وتقييم علاقتها بداء المقوسات في النساء الحامل والمجهضات. وجد أن وجود اثنين من تعدد اشكال النيوكليوتيدات المفردة في المنطقة المحركة للجين المعني هما rs2275913 من انترلوكين–17Aو rs4073 لانترلوكين–8 . تُظهر الأنماط الجينية الطافرة المتجانسة AA توزيعًا أكبر في مرضى داء المقووسات والنساء الحوامل المجهضات, مقارنة بالمجموعة الضابطة، وتدعم قيم نسبة الأرجحية (OR) أن الأليل A في AA مرتبط بالمرض محل الاهتمام ويعتبر أليل خطر. نسبة الأرجحية أعلى في النمط الجيني (0.40-7.3)1.7=AA مع قيمة P ذات دلالة عالية تبلغ ٠.٠٤، وتكرار الأليل A أعلى =(30%)12 في مجموعة المرضى الذين يعانون من ارتفاع نسبة الأرجحية (OR)= (0.61-4.8) 1.71 والقيمة P = 0.3 بينما تكرار الأليل هو (20%)8 منخفض في المجموعة الضابطة. تظهر نتائج محاذاة كروماتوكرام متعددة لمرضى التوكسوبلازما والنساء الحوامل المجهضات ثلاثة أنماط وراثية، AA و AG وGG. أليل A يعتبر أليلًا متحورًا يعتمد على SNP rs2275913الصحيح. تظهر الأنماط الجينية AG و AA توزيعًا أكبر في داء المقوسات ولمرضى من النساء الحوامل والمجهضات مقارنة بمجموعة التحكم. تدعم قيم نسبة الأرجحية (OR) ارتباط الأليل A

في كل من AG و AA ب المرض محل الاهتمام. يكون OR أعلى في الأنماط الجينية AG (0.019-52.03)1= ، قيمة P =1 و (0.40-7.3)1.7= AA مع قيمة P ذات دلالة عالية تبلغ (0.4).

تظهر النتيجة النهائية أن تحليل تعدد الأشكال للإنترلوكين-A 17وانترلوكين-8 له تأثير واضح على أي أمراض بشرية. في هذه الدراسة، أظهر تعدد الأشكال للإنترلوكين-17A وانترلوكين-8فعالية عالية في حدوث داء المقوسات في النساء الحوامل والمجهضات، ويظهر دور وتعدد الأشكال وتوزيع الإنترلوكين- 17A للأنماط الجينية rs2275913 G>A والإنترلوكين-8 rs4073 A>T . لا يتشابه بين المرضى والسيطره.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل – كلية العلوم للبنات

قسم علوم الحياة

ظاهرة تعدد الأشكال لأنترلوكين-8 و أنترلوكين-17A المرتبط بداء المقوسات في النساء الحوامل والمجهضات في محافظة بغداد

رسالة مقدمة الى

مجلس كلية العلوم للبنات، جامعة بابل

وهي جزء من متطلبات نيل درجة الماجستير علوم

في علوم الحياة

من قبل

مريم جبار كريم فرحان

(بكالوريوس علوم، علوم الحياة ، جامعة بابل ،٢٠١٧)

بإشراف

أ.د. احمد خضير الحميري

شعبان١٤٤٤ هـ

أذار ٢٠٢٣ م