



**University of Diyala**  
**College of Medicine**  
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# **Seroprevalence of Anti-Toxoplasma Antibodies in Patients Undergoing Hemodialysis in Diyala / Iraq**

**A Thesis**

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

يَا أَيُّهَا النَّاسُ قَدْ جَاءَكُمْ مَوْعِظَةٌ مِنْ رَبِّكُمْ  
وَشِفَاءٌ لِمَا فِي الصُّدُورِ وَهُدًى وَرَحْمَةٌ لِلْمُؤْمِنِينَ ﴿٥٧﴾

صدق الله العظيم

سورة يونس الآية (٥٧)

# DEDICATION

**TO** The Who longs to make our eyes close to seeing him... our beloved and our intercessor on the Day of Resurrection ... the master of the prophets and messengers ... our mister Muhammad (may God bless him and grant him peace) . **TO** The one who left our world and still has the sound of his advice guiding me ... my dear father . **TO** My paradise in this world ... to my straight path is the path of guidance ... to the fountain of love, patience and hope .... my beloved mother . **TO** My soul mate and path mate... to whom I walked the path step by step and accompany her until now... My dear sister Heba . **TO** My bond and my support after God... to the example of giving, pride and sacrifice... My brothers. **TO** I dedicate this work to the souls of the scholars of our nation, all the dead Muslims and innocent martyrs.

**Maysaloon**

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**I would like to start with gave the Almighty saying: (He who gives thanks will give thanks to himself) (Luqman, 12)**

**In recognition of the grace and adherence to the saying of the Messenger of God "He who does not thank people, does not thank God "**

**I thank God Almighty a lot, good and blessed filling the heavens and the earth for what he has honored me in completing this study, which I hope will satisfy him. Then I extend my sincere thanks and great gratitude to the University of Diyala / college of Medicine, which gave me the opportunity for scientific research, and I extend my thanks and gratitude to the consultant Dr. Nabil Khaled and Dr. Muhammad Jassim for their acceptance of supervising this thesis, and the follow-up, diligence and encouragement that had a great impact on its completion and its final appearance, so may God reward them the best reward for their efforts and bestowed upon them with more knowledge, which they did not spare me with advice or guidance throughout the period of conducting the research, I extend my thanks and gratitude to the assist.lec.hiba hadi rashid for her support and valuable guidance , and all thanks to istbraq fouad awad for her sound guidance and practical guidance, and thanks are extended to my family, who had a great role in completing the search . I thank Professor muhannad muhammad abdul sattar for his**

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## Summary

*Toxoplasma gondii* is the most common infection of protozoa, affecting a wide range of hosts. Toxoplasmosis is usually asymptomatic in immunocompetent persons, but in immunocompromised persons (ex patients on dialysis) significant problems can ensue, and may progress to a life-threatening infection. Our study was conducted to investigate the seroprevalence of anti-toxoplasma antibodies in patients undergoing hemodialysis in Diyala / Iraq . A group of blood samples, consisting of 75 dialysis patients and 50 healthy controls, totaling 125 were examined for the detection of anti-*toxoplasma* antibodies in Diyala Province from November 2020 to April 2021 using enzyme-linked immunosorbent assay (ELISA) for IgG and IgM. Of the 125 samples examined, 32 (42.67%) and 2 (4.0%) were IgG-positive for both patients and healthy individual respectively, and the results of the current study showed only 1 (1.33) was seropositive for IgM, while healthy people were not found seropositive for IgM . The results showed that the percentage of seropositivity was higher in females (45.45%) than males (40.47%) in the group of patients, but the differences were not statistically significant. As for the age groups, a positive increase was observed in the age group (11-30) years when comparing patients and healthy people, as the percentage reached (29.41%). It was also observed that the highest seropositivity in the education levels was in the group of patients at the educational level primary (45.45%) than in the healthy control group, but the differences were not statistically significant between education levels. Patients living in urban areas had significantly higher serum (P = 0.0041)

than patients in rural areas, and the odds ratio was (22,000). Regarding the effect of socio-economic factor on antibody serotype, the results showed that patients with average level showed higher seropositivity significantly ( $P = 0.0003$ ) than healthy subjects, with significant differences in socio-economic level . As for the dialysis time, hemodialysis twice a week showed serum positivity slightly higher than their counterparts (0.0068) , where the odds ratio was (0.8095) and confidence intervals (0.014-45.22) , and this indicates that there are significant differences in the number of dialysis times between patients. And those who had contact with cats showed a significant increase in seropositivity ( $p = 0.0001$ ) and odds ratio (20.5789), and no significant differences were observed between non-workers and workers. Besides, there were no significant differences in visual or hearing impairment, or both. It was also observed that there was a significant increase in seropositivity ( $P = 0.0002$ ) and odds ratio 0.8140 and CI 95% (0.015-43.147) . also this study were showed that the Mean  $\pm$  SE for Cystatin-C in the group of patients was ( $2.43 \pm 0.10$ ) higher compared to the healthy individuals ( $2.02 \pm 0.21$ ) , it shows the results of the correlation coefficient between *Toxoplasma* IgG and Cystatin-C in hemodialysis patients, where it was found that the correlation coefficient between them was (0.34) . Conclusions : dialysis patients are at risk for toxoplasmosis and should be screened on a regular basis to prevent the disease from spreading during hemodialysis.

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### Abbreviations Table

Abbreviations	Word
<i>T-gondii</i>	<i>Toxoplasma gondii</i>
TLR	Toll-like receptor
ELISA	Enzyme- linked Immunosorbent assay

<b>LAT</b>	<b>Latex Agglutination</b>
<b>ELA</b>	<b>Enzyme Immunoassay</b>
<b>IFA</b>	<b>Indirect Fluorescent Antibodies</b>
<b>IgG</b>	<b>Immunoglobulin G</b>
<b>IgM</b>	<b>Immunoglobulin M</b>
<b>AIDS</b>	<b>Acquired Immune Deficiency Syndrome</b>
<b>HIV</b>	<b>Human Immunodeficiency Virus</b>
<b>CKD</b>	<b>Chronic Kidney Disease</b>
<b>TH1</b>	<b>T helper 1</b>
<b>IFN</b>	<b>Interferon</b>
<b>DCs</b>	<b>Dendritic Cell</b>
<b>NK</b>	<b>Natural Killer</b>
<b>ILC1</b>	<b>Interleukin C1</b>
<b>CSF</b>	<b>Cerebrospinal Fluid</b>
<b>FAD</b>	<b>Food and Drug Organization</b>
<b>OR</b>	<b>Odds Ratio</b>
<b>CL</b>	<b>Confidence Interval</b>
<b>NP</b>	<b>Number Positive</b>
<b>NT</b>	<b>Number Tested</b>
<b>ER</b>	<b>Endoplasmic Reticulum</b>
<b>GFR</b>	<b>Glomerular Filtration Rate</b>
<b>HD</b>	<b>Hemodialysis</b>
<b>PD</b>	<b>Peritoneal Dialysis</b>
<b>CAPD</b>	<b>Continuous Ambulatory Peritoneal Dialysis</b>
<b>CCPD</b>	<b>Continuous Cycling Peritoneal Dialysis</b>
<b>IPD</b>	<b>Intermittent Peritoneal Dialysis</b>
<b>AKI</b>	<b>Acute Kidney Injury</b>

<b>CRRT</b>	<b>Continuous Renal Replacement Therapy</b>
<b>CF</b>	<b>Calibrator Factor</b>
<b>OD</b>	<b>Optical Density</b>
<b>AB</b>	<b>Antibodes</b>
<b>CRD</b>	<b>Chronic Renal Disease</b>



# **CHAPTET ONE**



## 1.1 Introduction

*Toxoplasma gondii* is an obligate intracellular parasite, a zoonotic disease, which causes the infectious disease toxoplasmosis. (Darde *et al* , 2011) *T. gondii* is found worldwide and can infect almost all warm-blooded animals. (Dubey, 2010) *T. gondii* is different from almost all other members of the Apicomplexa family (Apicomplexa) . This is because there are many types of host animals in the middle life cycle. (Kim *et al*, 2020)

However, felines like home cats, they are the only parasite hosts known to reproduce sexually. (Knoll, 2019) Infection does not cause clinical disease in most animal species, were it is estimated that about 40% of cats in the United States are infected. Most do not show any symptoms, but they may develop jaundice or blindness and experience personality changes if the parasite spreads to the liver or nervous system , (Razzaq , 2019) and it can cause acute, life-threatening diseases. ( Hill *et al* , 2005 )

*T.gondii* is one of the most common parasites in people in developed countries . (Flegr , et al, 2014) According to serological research, 30–50% of the world population has been exposed to and may be chronically infected with *T. gondii*, but infection rates vary significantly by country. (Falagas , *et al*, 2009 ) in the united states , it is estimated that 16–40% of the population are infected, whereas in Central and South America and continental Europe, estimates of infection range from 50 to 80% . ( Dubey and Baeatti,1988)

The sign and symptoms of this condition are quite changing, ranging from asymptomatic to severe infection . ( Dubey *et al*, 2012 ) This variation depends on several factors including virulence of the strain of toxoplasma, the individual's genetic background and the status of the immune system of the infected individual . (Montoya and Liesenfeld , 2004) In addition, since the organism has an affinity for muscular and neural tissues as well as the other



visceral organs, many hosts harboring latent tissue cysts vassal for toxoplasmosis . (Dubey *et al* ,1998 )

Toxoplasmosis is as a life-menacing risk infection in immunocompromised people. (Navia *et al*, 1986) Uremic patients have impaired immune responses, both cellular and humoral. (Vanholder *et al* , 1993 ) Because of the decreased circulating T-cells and increased suppressor cells, the immune status in CRF cannot be restored . (Glorieux *et al* , 2009) These factors are likely to contribute to acquired immune suppression in uremia and the high prevalence of infection among dialysis patients, as infection is a common and major cause of death in end-stage renal disease. ( Schollmayer and Bozkurt,1988) *Toxoplasma gondii* in humans is one of the most common parasites in the world , and infection usually occurs after ingestion of the unpopulated Oocyte, which are only shedded by cats. (Dubey, 2006 ) The oocysts in the external environment may contaminate soil, water, and vegetables, which is a major source of infection . (Lass et al, 2009) Other transmission routes include consumption of tissue bags in undercooked meat of an infected animal, organ transplantation, and through blood transfusion . (Torgerson and Mastroiacovo,2013)

In the 1930 and 1940 , scientists recorded that *Toxoplasma* had dangerous effects on individuals with compromised immune systems leading to blindness, brain lesions, and abscesses. Although this disease is widespread it has received little attention because it remains latent, in most humans (only 10-20% of infected people develop symptoms) . (Razzaq, 2019)

*Toxoplasma* has also been shown to make people reckless and less fearful, making people more likely to get involved in car accidents. Although the infection remains latent in humans, it has been shown to cause some behavioral changes, as a study in 2002 showed a link between car accidents

and *Toxoplasma* infection, and the study reported that people with infection were more involved in car accidents than their healthy counterparts . (Razzaq , 2019)

It was found that *toxoplasma* infection increases the likelihood of developing psychiatric disorders, such as schizophrenia. *Toxoplasma* deals with dopamine levels in the brain, because it contains an enzyme that can produce dopamine. Dopamine is a key neurotransmitter in movement and fear processing, giving a clear explanation for behavioral disturbances in humans as well as affected animals . (Razzaq , 2019)

In 1970 the life cycle of *T. gondii* was described (Veeranoo *et al* , 2017) which consisted from a sexual cycle in felidae and asexual cycle in all other warm blooded animals and as well human. (Bucko and Gieger , 2001) This asexual cycle is divided into two stages: tachyzoites, which multiply rapidly in a variety types of host cell , and bradyzoites, which multiply slowly in latent tissue cysts, have a high affinity for muscular tissues and neurological , and are found primarily in the central nervous system, the skeletal , and the eye and cardiac muscles. The chronic phase of toxoplasmosis is distinguished by the presence of Bradyzoites according to Dubey (1998a,b ) , while infected cysts can form in any visceral organ, including the kidneys, lungs , and liver . Tachyzoites are loaded into host cells and killed by their release, while bradyzoites trapped in tissue cysts continue to multiply via endodyogeny.(Tenter, 2009)

Seroepidemiological studies have shown that antibodies to *Toxoplasma gondii* are present in the serum of one third of the world's adult population. The prevalence of *Toxoplasma gondii* varies with age, humidity, and geographical location of the patient's eating habits. Serological research results indicate that toxoplasmosis is the most common human infection in

many parts of the world. The clinical signs and symptoms depend on the immune status of the host. In people with healthy immune systems, self-limiting infections will manifest themselves. People with compromised immune systems, particularly those with compromised cellular immune systems, are at danger of repetition or spread of infection. (Macpherson et al, 2005)

The Presence of *T.gondii* antibodies in a single serum sample only proves that the host was infected at some point in the past . So two samples were taken from the same person, with the second sample collected two to four weeks after the first sample. If the antibody titer in the second sample increased 16-fold, indicating an acute infection. High antibody titers may persist for several months after infection . The increase in antibody titers may not be related to clinical symptoms, because most human infections are asymptomatic. (Hill and Dubey , 2002)

Hemodialysis is a treatment to filter wastes and water from blood . Hemodialysis helps control blood pressure and balance important minerals, such as potassium, sodium, and calcium, in blood . (Hall *et al* ,2012)

Dialysis patients suffer an increased incidence of infections with toxoplasmosis , inferring that uremic patients' innate defense systems are impaired. Microbial recognition and subsequent release of proinflammatory cytokines are critical components of the innate host defense system against microorganisms. Members of the Toll-like receptor (TLR) family are responsible for recognizing pathogen-associated molecular patterns expressed by a wide range of infectious agents. TLRs modulate cytokine expression by activating the nuclear factor-kB pathway via several adaptor molecules. Activation of the nuclear factor-kB pathway connects the innate and adaptive

immune responses via the production of inflammatory cytokines and the inducement of costimulatory molecules. (Ando *et al* , 2006 )

Since hemodialysis patients are at a higher risk of *T. gondii* infection thus, the aim of our study was to investigate seroprevalence of toxoplasmosis in hemodialysis patients of Diyala , in 2020-2021 .On the other hand, toxoplasmosis occurs mostly in tropical and subtropical regions (Dubey ,2008) .

## **1.2 Aims of the study**

Our current study aims to :

1-Detect the rate of infection with *Toxoplasma gondii* among patients hemodialysis .

2-Determine cystatin-c level , IgG and IgM anti-*T. gondii* antibodies in hemodialysis patients.

3- Study the effect of different parameters such as age, gender, cat breeder , other disease associated with *T. gondii* infections .

4-Study is to look into the seroepidemiology of *Toxoplasma gondii* in dialysis patients Baquba / Iraq .